Oestrogenicity and chemical target analysis of water from small-sized industries in Pretoria, South Africa

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Abstract

Increasing concern about endocrine disrupting chemicals (EDCs) and their effects on humans, animals and the environment resulted in this study being conducted. Water from 7 sites in the Pretoria West area (South Africa), with significant numbers of small-sized industries, was screened for oestrogenicity, using the Recombinant Yeast Cell Bioassay (RCBA). Target chemical analyses were carried out to establish the presence of EDCs, including p-nonylphenol (p-NP), bisphenol A (BPA), phthalate esters, polychlorinated biphenyls (PCBs) and various organochlorine pesticides, including dichlorodiphenyltrichloroethane (DDT). p-NP, PCBs and organochlorine pesticides were detected using LECO Pegasus II MSTOF and BPA and phthalates were detected using the GC-MS method. Oestrogenic activity was detected in all the samples collected from these sites. Lindane, an organochlorine pesticide, was detected at one site. p-NP, PCBs and phthalate esters were detected at some of the other sites. Small-size industries were found to contribute to EDC pollution of water in the Pretoria West area.

Keywords: oestrogenicity, endocrine disrupting chemicals (EDCs), p-nonylphenol (p-NP), polychlorinated biphenyls (PCBs), bisphenol A (BPA), phthalate esters, organochlorine pesticides, dichlorodiphenyltrichloroethane (DDT), lindane

Introduction

Over the past two decades, increasing concern and public debate have developed over the potential adverse effects of exposure to a group of chemicals that have the potential to alter the normal functioning of the endocrine system in wildlife and humans (Sharpe and Skakkebeak, 1993; NIEHS, 2006). A large number of these are man-made and are present in the environment as pollutants (Sharpe and Skakkebeak, 1993). These chemicals are known as endocrine disrupting chemicals (EDCs) since they act like oestrogens or mimic or suppress the action of hormones, particularly oestrogen (McLachlan and Arnold, 1996).

EDCs are found in many everyday products including some plastic bottles, metal food cans, flame retardants, toys, cosmetics, pesticides and detergents (NIEHS, 2006). A wide and varied range of chemicals are thought to cause endocrine disruption. These include diethylstilbestrol (DES), polychlorinated biphenyls (PCBs), dioxin and dioxin-like compounds, organochlorine pesticides including dichlorodiphenyltrichloroethane (DDT), alkylphenols and phthalate esters (EDSTAC, 1998; NIEHS, 2006).

The key health impacts that have been attributed to EDCs include abnormalities in male reproductive health (Toppari et al., 1996; Kamrin, 1996; De Jager et al., 1999; De Jager et al., 2001; Rozati et al., 2002; Aneck-Hahn et al., 2007; Kilian et al., 2007); female reproductive health (Sharpe and Skakkebeak, 1993; Kirkhorn and Schenker, 2002); increased incidences and accelerated progression of cancers (Sharpe and Skakkebeak, 1993); immunological effects (Porter et al., 1999; WHO, 2002); and neurodevelopment impacts (Eskenazi et al., 2006). Recent research published by Kaiser et al. (2005) suggests that pesticides and other man-made chemicals may lower male fertility for at least 4 generations.

Male reproductive health

Concern is growing that abnormalities in male reproductive health are becoming more frequent. There has been an increased frequency of testicular cancer and of boys born with urethral abnormalities and undescended testes. Sperm counts have also declined by about a third in the past 20 years, at a rate of about 2.1% per year, and the quality of sperm has declined as well (Lutz, 1996; Swan et al., 2003; Aitken et al., 2004; Swan, 2006).

Studies have identified EDCs as causing infertility and behavioural changes in species such as polar bears, beluga whales, alligators and humans (Colborn et al., 1993; Kamrin, 1996). Relevant research in South Africa using environmental toxicants with oestrogenic properties has also shown structural changes in the reproductive system of male rats that have resulted in decreased fertility parameters (De Jager et al., 1999; De Jager et al., 2001; Kilian et al., 2007). This finding is supported by the first histological evidence of intersex in feral sharptooth catfish from an oestrogen-polluted water source in Gauteng, South Africa. Target chemical analyses showed that the water, sediment, and serum samples tested positive for p-NP (Barnhoorn et al., 2004).

In South Africa research in this area is ongoing. Research undertaken by the University of Pretoria on the effects of p-NP and on mixtures of EDCs, including phytoestrogens, has shown a general trend in decreased fertility parameters in adult rats (Kilian et al., 2007). Semen quality with environmental DDT exposure in young men living in a malaria area in the Limpopo Province of South Africa showed impaired seminal parameters. The high exposure levels of DDT and p,p'-dichlorodiphenyl-

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dichloroethylene (p,p'-DDE) are of concern because these levels could have far-reaching implications for reproductive and general health (Aneck-Hahn et al., 2007).

Female reproductive health

In women the incidence of breast cancer in Western Europe and the USA has increased since 1940 and breast cancer is the most common cancer found in women in these countries. Endometriosis, a formally rare but painful and disabling disease affecting women, which could lead to infertility problems, has been recently found to affect 5 million women in the USA alone (Kirkhorn and Schenker, 2002).

Immunological effects

Diethylstilbesterol (DES), PCBs and dioxins have been shown to alter human and animal immunity. Specifically, DES was found to cause a weak immunological change following *in utero* exposure, while PCBs have been reported to alter immune param-

TABLE 1
Detergents used by small-sized industries in Pretoria West, as determined by questionnaire

Industry	Detergents	Area to be cleaned
Pie Manufacturer	Oven cleaner, heavy-duty pine gel, no name dishwasher, toilet bowel cleaner, Jeyes fluid	Ovens; floors; dishes; toilets; drains
Dup's Scrapyard	Sunlight liquid, Harpic, Handy Andy, grease-cutter, Omo, multipurpose cleaner, hand cleaner (wholesalers)	Dishes, toilets, engines, web machine, car body; washing hands
Tip Top Café	Persal (Trade Centre)	Floors, ovens, dishes, etc.
Claudinos Pizza	Pan release (Chipkin, Jhb), green dishwashing liquid (Cater sales), Javel	Pans, dishes, sanitisation of surfaces
Early Bird Service	Service sol, surgical spirits, commercial detergents	Switch cleaner, floors
D & J Service Station	Handy Andy, no name soap powder, Econo liquid wax and wash, commercial window cleaner	Floors, driveway, cars, windows
Magic Photos	Chemical cleaners (Photo Ease Chem Cor product), Wonderclean, Mr Muscle	Grease on photographic and printing machine, floors, counters and windows
T & M Motor Repairs and Services	Hand cleaner (No-name brand), engine cleaner	Cleaning of hands, engines and floors
Mr Cash and Mr Valet	Connex, shampoo, heavy duty cleaner	Engines, car body, car seats
Church Street Motors	Engine cleaner (no name brand), car wash gel, hand cleaner	Engines, cars, hand washing
Precision Autobody	Car wash and wax (Quality clean), washing powder, floor cleaner	Washing and waxing, car body, floors
Supercare Autobody	Household liquid soap, Handy Andy, Jik, diesel	Car body, floors, engine grease removal
Sao Tiago Café	Family Favourite (Drug Centre), washing powder	Fryers, pans, oven, floors
Enzos Panel Beaters	Sunlight Liquid, steam cleaning, valet	Car body, engines, interior of cars
All Power	Momar products or sales	Grease from machinery
Lusio Autobody Repairs	Omo/Surf, car sprays and cockpit sprays (Makro)	Car body and grease, interior of cars, rims, tyres
Eljoney Executive Body Repairs	No-name brand green liquid (bought from city centre)	All purpose
Precision Autospray	Car Shampoo (Marie Daniel Chemochem, Onderstepoort), action engine cleaner, Harveys	Interior and exterior, grease removal
Body and Panel	Engine cleaner, washing powder, car shampoo (Marie Daniel Chemochem, Onderstepoort), hand cleaner	Engines (multi-maintenance products), interior, hand washing, car body
Dirks Panel Beaters	Household washing powder, PH polish	Car body, polishing of cars
Jacaranda Panel Beaters	Surf, Valet	Car body, bigger jobs
Progress Panel Beaters	Safic dishwasher	All-purpose wash
Gilos Panel Beaters	Car wash and wax, engine cleaner, hand cleaner, silicone spray	Washing and waxing of cars (Marpro Zap), grease removal, washing of hands, dashboard
Italian Panel Beaters	Liquid clean (quality clean), engine cleaner	Car body, engine
Toria Panel Beaters	Multipurpose cleaner (Rodita Manufacturers), car wax, dishwashing cleaner, stripper (Harveys)	Car body, waxing exterior, general cleaning, strong degreaser
Competition Motors	Will Penn Engine Cleaner	Engine grease removing
Competition Motors	Dishwashing liquid	Car body
Landmans Garage	Washing powder (no name brand), engine detergent	Car body, engine cleaner
Western Bikes	Engine cleaner (Spare Shops), paraffin	Engine grease removing, stripping of grease
Valhala Service Station workshop	Will Penn auto lubricants, Prepsol degreaser	Engine degreaser, floors
Invector Autobody	Sunlight Liquid, degreaser (Viva Spares)	Car body, grease removal
Targa Panel Beaters	Dishwashing liquid (Zircon Cleaning Products), Spot remover/cleaner, engine cleaner (One-Stop Motors)	Car body, interior of cars, grease removal from cars
Dairy World	HCl, caustic soda, hydrogen peroxide, chlorine and Jik, iodine, steam	Pasteurising milk containers, cleaning of machinery and pipes where milk runs through, table surfaces in the process plant, sanitising plastic drums and lids, rinsing of pipes and washing of hands, sterilisation

eters following accidental, occupational, and general population exposures (WHO, 2002).

Neurodevelopment impacts

Research shows that endocrine disruptors may pose the greatest risk during prenatal and early postnatal development when organ and neural systems are developing (NIEHS, 2006). A longitudinal birth cohort study of predominantly Mexican American females residing in California provided evidence that *in utero* exposure to DDT and, to a lesser extent, p,p'-DDE is associated negatively with childhood neurodevelopment. Although animal studies have demonstrated that DDT is a neurodevelopmental toxicant, the present study is the first to report on DDT and neurodevelopment in humans (Eskanazi et al., 2006)

Water

In many developing countries, less than a quarter of the population has adequate waste disposal systems and clean drinking water. In South Africa, a country with rapid urbanisation and industrial growth, the number of small-sized industries is growing fast (Jeyaratnam, 1992). This growth implies that industrial, household and agricultural waste is increasing, which poses a problem as the proper disposal of these pollutants is limited. Often, this complex mixture of toxic compounds and pollutants is disposed of into surface waters, such as dams, rivers, and eventually the sea. These toxic contaminants may disturb the biological conditions of aquatic ecosystems and be harmful to humans, if they end up in food or drinking water (De Jager et al., 2002; Aneck-Hahn, 2003).

An audit of chemical products used in small-sized industries in Pretoria West highlighted the significant use of detergents. However, the lack of adequate legislation on the use, handling and disposal of such chemicals for small-sized industries creates a potential hazard for workers, the surrounding communities and the environment (Jeyaratnam, 1992).

In the area investigated in the current study, the wastewater from industry and households, as well as sewage, is treated at the Daspoort Wastewater Treatment Works plant, via biological and physico-chemical means, before being released into the Apies River, which feeds into the Bon-Accord Dam and finally into the Pienaars River. While this treatment reduces the concentrations of viruses, bacteria and biological substances, it often does not eradicate all the chemicals, including those synthetic and natural oestrogens that have accumulated in the organic matter and in some instances may even result in the introduction or accumulation of these EDCs (Muller et al., 2004).

The aim of this study was to screen for oestrogenic activity and to test for specific target chemicals most likely to be used or produced as by-products in the small industries in the Pretoria West area.

Methodology

This was a descriptive study and was carried out in two phases. Phase 1 involved the identification of the different types of industries and the use of industrial detergents and other cleaning agents in the various processes by using a questionnaire (Table 1). Phase 2 involved water sampling and chemical analysis (Table 2 & 3).

The study area is approximately 2.6 km x 1.8 km, and is situated south of Church Street, west of DF Malan Street, east of Buitekant Street, and north of the railway line. This area was specifically demarcated as it comprises the greatest number of small-sized industries in the Pretoria West area.

Survey of industries in the study area

Industries in the area were surveyed by means of a questionnaire, distributed to all the industries in the area that employ fewer than 50 workers. More than 70% of the industries responded by completing the questionnaire as requested. Industries that did not complete the questionnaire had either closed down or the manager/owner of the establishment was not available at the time of the interview. Industries in this area comprise mainly food retail outlets, panel beaters, auto-body repair works, auto-spray painting services and several general dealers.

Sample collection

In Phase 2, water samples were collected on a once-off basis from 7 different points in the study area (Table 2). Test points were selected in consultation with city engineers from the Water and Consumer Management Division of the Tshwane Department of Water and Sanitation. The criteria for selection included points where water converges from the high-risk industries in the study area. These are the most northerly points, i.e. the water moves in a northerly direction towards the Daspoort Wastewater Treatment works.

TABLE 2
Identification and description of water-sampling sites in Pretoria West

Sample collection point	Location of collection point	Description of the area where sample was collected
1	Corner of Buitekant and Church Streets	Dense small industries, namely motor service and repair shops, a petrol station and several food outlets.
2	Corner of Rebecca and Church Streets	This area has mainly small food outlets.
3	Corner of Zieler and Church Streets	This area is again a very dense industrial area. A manufacturing plant, where yoghurt and other dairy products are manufactured, is situated in this area and water from this area drains into these two underground points.
4	Corner of Zieler and Church Streets (opposite end of collection point 3)	
5	Corner of President Burger and Church Streets	Slightly less dense area than the rest. There are a few households and some light industries in this area.
6	At the entrance of Daspoort Wastewater Treatment Works	At this point effluent converges from all the industries, and from the few households in this area.
7	Point where effluent enters the Apies River	The water at this point has been cleaned and can be considered safe for drinking and other uses

TABLE 3
Oestrogenic activity expressed as EEQ and target chemicals determined in water samples collected in Pretoria West

Sample	Oestrogenic activity g/ℓEEQ (±SD)	Organochlorine pesticides µg/ℓ	p-NP µg/ℓ	DEHP µg/ℓ	DBP µg/ℓ
1	Toxicity detected	-	119	35	7
2	9.48×10^{-13} ($\pm 8.05 \times 10^{-12}$)	-	-	15	-
3	8.16×10^{-13} ($\pm 3.96 \times 10^{12}$)	Terbutylazine (not quantified)	10	5	4
4	5.8×10^{-9} ($\pm 1.25 \times 10^{-8}$)	-	-	41	5
5	1.28×10^{-11} ($\pm 2.9 \times 10^{-12}$)	-	20	47	-
6	2.44×10^{-9} ($\pm 3.4 \times 10^{-8}$)	-	-	69	11
7	2.37×10^{-11} ($\pm 3.28 \times 10^{-12}$)	Lindane - 0.9 Atrazine (not quantified) Chlorpyrifos (not quantified)	10	5	-

The water samples were collected in 2 x 1 ℓ glass bottles according to standard procedures (Aneck-Hahn, 2003). The bottles were washed in chromic acid and rinsed with ethanol and methanol to ensure that there were no particles that could contaminate the sample. A foil cover was used, followed by a plastic bottle top. The foil cover prevents the plastic top from leaching EDCs into the water and contaminating the sample. At each sample point, a stainless-steel beaker was suspended by a rope and lowered into the specifically selected manhole and water was collected into it and poured into a marked glass bottle. The beaker was rinsed with ethanol before being lowered at each site to minimise cross-contamination of water. The samples were collected in February on a Friday, in the later part of the day, as this is usually the time of day and week when most industries clean their equipment, plants or sites. All the water samples collected were then stored at ±5^{0}C until analysed.

RCBA bioassay

The RCBA was carried out according to the method developed by Routledge and Sumpter (1996) and modified by Aneck-Hahn et al. (2005). The oestrogenic activity was compared to that of 17ß-estradiol (E_2), a natural oestrogen used as a positive control in *in vitro* assays.

After 4-day incubation the endpoint measured in this assay was ß-galactosidase activity. For determination of estrogenic activities, 200 µℓ of the water samples extract was added to the first column of a 96-well plate (Cat. No. 95029780, Labsystems) and then diluted in a 1:2 series across the assay plate (12 dilutions). Ethanol was used as a negative control. A standard curve for 17β-estradiol (Cat. No. E8875, Sigma), ranging from 1×10^{-8} M to 4.8×10^{-12} M (2.274×10^{-6} g/ℓ to 1.3×10^{-9} g/ℓ) which was extended to a lower concentration of 1.19×10^{-15} M (3.24×10^{-13} g/ℓ) was included on each plate (positive control). Data were expressed as the mean of two replicates. Based on the dose-response curves for E_2 and the test sample, the estradiol equivalent (EEQ) of each sample was calculated using the EC_{50} value (absorbance) of the sample. The EEQ concentration was then adjusted with the appropriate dilution factor of the sample (i.e. concentration factor of 1000 x) (Aneck-Hahn, 2003).

Target chemical analyses

Target chemical analysis was carried out to detect PCBs, BPA, p-NP, phthalate esters; di-(2-ethylhexyl)-phthalate (DEHP), di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), and the organochlorine pesticides. All solvents used were of pesticide trace analysis grade. One litre of the water sample (containing 2% methanol) was passed through a pre-conditioned C18 solid phase extraction cartridge or column. The residue was reconstituted with 1mℓ hexane and vortexed before it was transferred to a vial (Naudé, 2002).

Single determinations using a South African Bureau of Standards (SABS) in-house method: AM178 A (WHO, 2003; Naudé, 2002) were used to test for p-NP, PCB and organochlorine pesticide residues in the water samples. A recovery determination was carried out by adding a known amount of pollutants to distilled water and analysing it concurrently with the samples, using a LECO Pegasus II MSTOF (Time of Flight Mass Spectrometer). Organochlorine pesticides and PCBs had a recovery range of 56 to 138% and 52 to 130% respectively. The recovery rate for p-NP is 10%. The limit of detection (LOD) for organochlorine pesticides and PCBs was 0.1 ug/ℓ and for p-NP it was 1 ug/ℓ (WHO, 2003; Naudé, 2002).

The phthalate ester and BPA determinations were done at the CSIR Biochemtek Laboratories, Johannesburg. A liquid-liquid extraction of the sample was done using dichloromethane as an extraction solvent. The phthalates were determined using an in-house gas chromatography-mass spectrometry (GC-MS) method AM 186 based on US EPA 8260 (US-EPA Method 8260C, 2006). The lowest LODs using this method are: DEHP at 4 µg/ℓ, DBP at 3 µg/ℓ and BBP at 4 µg/ℓ. The same analytical procedure was followed, to analyse for bisphenol-A although this chemical is not a target analyte for the EPA 8270 method. Identification of the target analytes was accomplished by comparing their mass spectra with the electron impact spectra of authentic standards. Quantification of each component was accomplished by comparing the relative response of a major ion relative to an internal standard and a minimum 5-point calibration curve (Garretson and Koning, 2001).

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Results

The results in this study showed oestrogenic activity and demonstrated measurable levels of chemicals recognized as EDCs in the different water sources.

Manholes

Water analysed from all 5 manholes in the study area (Table 3) showed oestrogenic activity, with EEQ levels ranging from 8.16×10^{-13} to 2.44×10^{-9} g/ℓ. Detectable concentrations of p-NP (10 to 119 µg/ℓ), DEHP (5 to 69 µg/ℓ), DBP (4 to 11 µg/ℓ) was also measured in all the water samples. The organochlorine pesticides detected included an unquantified amount of terbutylazine (TBA). No PCBs or BBPs were detected in any of these waters.

Manhole where all the water from this area converges

The water sample from this manhole (Sample 6) showed oestrogenic activity (2.44×10^{-9} g/ℓ EEQ), and measurable levels of DEHP (69 µg/ℓ) and DBP (11 µg/ℓ). This water sample did not show detectable levels of organochlorine pesticides or p-NP compounds (Table 3).

Exit from the Daspoort Wastewater Treatment Works

The water sample (Sample 7) collected after processing in the Daspoort Wastewater Treatment Works, before it enters the Apies River, showed oestrogenic activity (2.37×10^{-11} g/ℓ EEQ). Target analyses confirmed the presence of p-NP (10 µg/ℓ), DEHP (5 µg/ℓ) and organochlorine pesticides, namely lindane (0.9µg/ℓ), and an unquantified amount of atrazine and chlorpyrifos (Table 3).

Discussion

In this study a once-off series of water samples, taken at the end of a week (Friday) when most small industries clean their equipment and sites, were screened for oestrogenicity and analysed for the detection of specific industrial chemicals known to be associated with EDC activity. The results show that the samples taken from the selected manholes and from the entrance and exit to the Daspoort Wastewater Treatment Works showed oestrogenic activity at varying EEQ levels (Table 3). Organochlorine pesticides were detected in water from two of the sample sites. The pesticide terbutylazine (TBA) was detected from the manhole at the corner of Zieler and Church Streets (Table 2). This pesticide was not quantified, as it does not form part of the list of organochlorine pesticides tested for. However, 0.9 µg/ℓ of lindane, and unknown quantities of chlorpyrifos and atrazine were detected at the entrance of the Apies River, after the water had passed through the Daspoort Wastewater Treatment Works.

Oestrogenic activity was detected in water samples from Manholes 2 to 5 and from the entrance (6) and exit (7) of the Daspoort Wastewater Treatment Works (8.16×10^{-13} to 5.8×10^{-9} g/ℓ). Cytotoxicity with no detectable oestrogenic activity was observed in Manhole 1. Some cytotoxicity was also detected together with oestrogenic activity in samples 3, 5 and 7. High levels of p-NP (10-119 µg/ℓ) in the water could account for the cytotoxicity observed. Some EDCs, including p-NP, are known to be responsible for the cytotoxicity observed in the RCBA assay (Bornman et al., 2007). The oestrogenic activity found in this study was higher than that measured in environmental samples taken from a peri-urban region near Pretoria (Bornman et al., 2007). These values also correspond with the study done by Matsui et al. (2000) in Japan. EEQ present in sewage treatment effluents ranged from 5 to 15 ng/ℓ and lake water 1 ng/ℓ.

All the chemical compounds tested for in this study were present, with p-NP and DEHP being the highest or most common. In 4 of the 7 manholes p-NP was detected. The latter is present in most plastic products and leaches into food and other consumables; it is also used as a carrier in many pesticides (Jobling and Sumpter, 1993; Melnick et al., 2002). No minimum guideline has been set in South Africa or by the EU on permissible or allowable levels in drinking water of p-NP or the nonylphenol group as a whole. This compound is capable of disrupting the endocrine system of animals, including humans and fish and therefore poses a health risk (Jobling and Sumpter, 1993).

The phthalates tested for were DEHP, DBP and BBP. However, no BBP was detected with the method used. Two of the manholes had various levels of DEHP, while DBP was detected in four of the manholes. The entrance to the Daspoort Waste Water Treatment Plant had the highest levels of both DEHP and DBP. This was the point where all the water from the entire Pretoria West area converges and seems to be the most DEHP and DBP contaminated point. DEHP is a general plasticizer in many PVC consumer products (Kavlock et al., 2002a). The range of exposure in the general population from all sources, excluding non-dietary ingestion, medical and occupational, is estimated to be 3 to 30 µg/kg body weight (Kavlock et al., 2002b). There are sufficient data in rodents to conclude confidently that oral exposure to DEHP can cause reproductive and developmental toxicity in rats and mice (Kavlock et al., 2002b; Duty et al., 2003). There are no minimal levels set for phthalates in drinking or other water.

The European Union (EU) directive for drinking and groundwater states that pesticide levels must not exceed 0.1 µg/ℓ for individual compounds and some of their degradation products and that the sum total of all pesticides should not exceed 0.5 µg/ℓ. The proposed guideline value for lindane in drinking water is 0.3 µg/ℓ as recommended by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) which conducted a recent review of lindane (WHO, 2003). In South Africa, lindane is being phased out for environmental use, and is now identified as one of the persistent organic pollutants (POPs) (WHO, 2003; UNEP, 1996).

As there are a few households in the area and many small industries that manufacture products, it is possible that lindane could have entered the underground water from both domestic and industrial use. Chlorpyrifos (used as an insecticide in homes and the garden) and atrazine (used to suppress weed growth in edible crop production) were not quantified.

Research into the effects of mixtures of oestrogenic chemicals has revealed the capacity for similar acting chemicals to act in combination, according to the principles of concentration addition (Brian et al., 2007). The levels and the combination of the EDCs detected in water samples from the Pretoria West area are therefore cause for concern.

Conclusions

It is predicted that small-size industries are contributing to pollution of the water in the Pretoria West area and, as a consequence, are polluting the waters of the surrounding areas.

Water in this study showed oestrogenic activity and specific individual chemical compounds known to have EDC activity were detected. At present there are no acceptable guideline values for the majority of EDCs tested. A dose-response relationship, particularly for extremely low exposure levels typical of environmental exposures, is difficult to establish (London et al., 2005). However, even low levels of individual chemicals and combinations of chemicals may pose a potential health risk to humans, animals and aquatic life (Bornman et al., 2007).

An activated sludge treatment process, which uses ultraviolet (UV) light and chlorine, is used to treat the water at the Daspoort Wastewater Treatment Works (Saayman, 2004). The process removes microbial agents, suspended particulate matter and some hazardous chemicals, but does not adequately remove pesticides and other EDCs. Only biodegradable chemicals are removed. There are processes that remove EDCs from wastewater, but these are sophisticated and very expensive (Arnot and Zahir, 1996). The Rietvlei Water Treatment Plant uses activated carbon, which removes certain EDCs and other chemicals (Muller et al., 2004).

Technologies such as increasing the sludge age of the effluent that passes through the water treatment works exist and are being looked at. Although these may not remove all the EDCs, they may assist in removing the slowly biodegradable organic matter, which most of the present water treatment systems do not do. In addition, the utilisation of membrane bioreactors to treat industrial and other waste, research on which has been done by the University of Bath, could be a breakthrough for South Africa in the near future (Arnot and Zahir, 1996).

At present, drinking and other water is not tested for these EDCs, except for some organochlorine pesticides (DWAF, 1996). For this reason, legislation for acceptable levels for these specific chemicals is not available. There is, however, a project underway that will consider updating and expanding the South African water quality guidelines and that may include EDCs, more specifically for water (Jooste, 2005).

Recommendations

Pollution of the water by under-regulated small-sized industry poses a serious threat to health, both to the workers in the industry and members of the surrounding communities. These hazardous exposures should be reduced, by the encouragement of small industry and government to jointly find safe, alternate means of disposing the waste and reducing exposure to various chemicals and finding enhanced technical processes for the treatment of domestic, agricultural and industrial wastewaters to support the reuse of water.

Pesticide pollution of water has received some attention in South Africa. However, South Africa still lags behind with monitoring data on which to base policy.

Lindane, an organochlorine pesticide, was detected in the water that had already passed through the Daspoort Wastewater Treatment Works. The level is above the acceptable daily intake limit for water (WHO, 2003) and poses a health risk for workers and the surrounding communities and further testing in this area is needed.

Financial and technical constraints, the shortage of skills for pesticide analyses, and the fragmentation of legislation across 14 Acts and 7 different government departments have resulted in weak regulation (London et al., 2005).

More specific regulations of EDCs are needed, particularly for water quality, where permissible levels of specific chemicals are clearly set.

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References

AITKEN RJ, KOOPMAN P and LEWIS SEM (2004) Seeds of concern. *Nature* **432** 48-52.

ANECK-HAHN NH (2003) Screening for Anti-Oxidant Pollutants and Estrogenicity in Drinking Water in Poverty Stricken Areas of South Africa. D. Tech. Thesis, Tecknikon Pretoria, Pretoria.

ANECK-HAHN NH, DE JAGER C, BORNMAN MS and DU TOIT D (2005) Oestrogenic activity using a recombinant yeast screen assay (RCBA) in South African laboratory water sources. *Water SA* **31** 253-256.

ANECK-HAHN NH, SCHULENBURG GW, BORNMAN MS, FARIAS P and DE JAGER C (2007) Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Limpopo Province, South Africa. *J. Androl.* **28** (3) 423-434.

ARNOT TC and ZAHIR N (1996) Membrane bioreactors as an alternative to conventional waste water treatment processes. *Resour. Environ. Biotechnol.* **1** 145-162.

BARNHOORN IEJ, BORNMAN MS, PIETERSE GM and VAN VUUREN JHJ (2004) Histological evidence of intersex in feral sharptooth catfish (*Clarias gariepinus*) from an estrogen-polluted water source in Gauteng, South Africa. *Environ. Tox.* **19** 603-608.

BORNMAN MS, VAN VUREN JH, BOUWMAN H, DE JAGER C, GENTHE B and BARNHOORN EJ (2007) The Use of Sentinel Species to Determine the Endocrine Disruptive Activity in an Urban Nature Reserve. WRC Report No 1505/1/07. Water Research Commission, Pretoria, South Africa.

BRIAN JV, HARRIS CA, SCHOLZE M, KORTENKAMP A, BOOY P, LAMOREE M, POJANA G, JONKERS N, MARCOMINI A and SUMPTER JP (2007) Evidence of estrogenic mixture effects on the reproductive performance of fish. *Environ. Sci. Technol.* **41** 337-344.

COLBORN T, VOM SAAL FS and SOTO A (1993) Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health. Perspect.* **101** (5) 378-384.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (1996) *South African Water Quality Guidelines.* **Vol. 1**: *Fresh Water* (2nd edn.) DWAF, Pretoria, South Africa.

DE JAGER C, BORNMAN MS and VAN DER HORST G (1999) The effect of p-nonylphenol, an environmental toxicant with oestrogenic properties, on fertility potential in adult male rats. *Andrologia* **2** 99-106.

DE JAGER C, BORNMAN MS, ANECK-HAHN N, FOURIE MH and BAILEY JL (2001) Additive Effects of p-Nonylphenol and Phytoestrogens on Reproductive Parameters in Male Rats. Poster Presentation, 2nd Int. Conf. Male Mediated Development Toxicity. 20-23 June, Montreal, Canada..

DE JAGER C, MYBURGH J, VAN DER BURG B, LEMMEN JG and BORNMAN MS (2002) Estrogenic Contamination of South African River Waters: A Pilot Study. American Waterworks Water Association (AWWA) April 18-20, Cincinnati, Ohio, USA.

DUTY SM, SILVA MJ, BARR DB, BROCK JW, RYAN L, CHEN Z, HERRICK RF, CHRISTIANI DC and HAUSER R (2003) Phthalate exposure and human semen parameters. *Epidemiol.* **14** 269-277.

EDSTAC (1998) Endocrine Disruptor Screening and Testing Advisory Committee, Final Report, EPA, Washington, USA.

ESKENAZI B, MARKS AR, BRADMAN A, FENSTER L, CAROLINE J, BARR D and JEWELL NP (2006) In-utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children. *Pediatrics* **118** (1) 233-241.

GARRETSON L and KONING S (2001) Analysis of semi-volatile organics according to EPA Method 8270. Veldhoven, The Netherlands. http://www.atasgl.com.

Available on website http://www.wrc.org.za
ISSN 0378-4738 = Water SA Vol. 34 No. 3 July 2008
ISSN 1816-7950 = Water SA (on-line)

JEYARATNAM J (1992) Occupational health services and developing nations. In: Jeyaratnum J (2nd edn.) *Occupational Health in Developing Countries.* Oxford Medical Publications. 3-30.
JOBLING S and SUMPTER JP (1993) Detergent components in sewage effluent are weakly oestrogenic to fish: An *in vitro* study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquat. Toxicol.* **27** 361-372.
JOOSTE S (2005) Personal communication. Resource Quality Services, Roodeplaat Dam, Department of Water Affairs and Forestry. Gauteng, SA.
KAISER J (2005) Endocrine disruptors trigger fertility problems in multiple generations. *Sci* **308** (5727) 1391-1392.
KAMRIN MA (1996) The mismeasure of risk: a review of 'Our Stolen Future'. *Scientific American*: *Reviews and Commentaries*. 1-4. Accessed on 20 October 2007. http://www.sciam.com/0996issue/0996review2.htm
KAVLOCK R, BOEKELHEIDE K, CHAPIN R, CUNNINGHAM M, FAUSTMAN E, FOSTER P, GOLUB M, HENDERSON R, HINGERG I, LITTLE R, JENNIFER S, SHEA K, TABACOVA S, TYL R, WILLIAMS P and ZACHAREWSKI T (2002a) NTP Centre for the Evaluation of Risks to Human Reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di-n-butyl phthalate. *Reprod. Toxicol.* **16** 489-527.
KAVLOCK R, BOEKELHEIDE K, CHAPIN R, CUNNINGHAM M, FAUSTMAN E, FOSTER P, GOLUB M, HENDERSON R, HINGERG I, LITTLE R, JENNIFER S, SHEA K, TABACOVA S, TYL R, WILLIAMS P and ZACHAREWSKI T (2002b) NTP Centre for the Evaluation of Risks to Human Reproduction: Phthalates expert panel report on the reproductive and developmental toxicity of di(2-ethylhexyl) phthalate. *Reprod. Toxicol.* **16** 529-653.
KILIAN E, DELPORT R, BORNMAN MS and DE JAGER C (2007) Simultaneous exposure to low concentrations of dichlorodiphenyltrichloroethane, deltamethrin, nonylphenol and phytoestrogens has negative effects on the reproductive parameters in male Spraque-Dawley rats. *Andrologia* **39** 128-135.
KIRKHORN SR and SCHENKER MB (2002) Current health effects of agricultural work: Respiratory disease, cancer, reproductive effects, musculoskeletal injuries and pesticide-related illnesses. *J. Agric. Saf. Health* **8** 199-214.
LONDON L, DALVIE MA, NOWICKI A and CAIRNCROSS E (2005) Approaches for regulating water in South Africa for the presence of pesticides. *Water SA* **31** 53-59.
LUTZ D (1996) Anecdotal evidence. No conception. Masquerading as sex hormones, chemicals ubiquitous in the environment could threaten our children's ability to reproduce. *The Sciences* **36** (1) 12-15.
MATSUI S, TAKIGAMI H, MATSUDA T, TANIGUCHI N, ADACHI J, KAWAMI H and SHIMIZU Y (2000) Estrogen andestrogenmimics contamination in water and the role of sewage treatment. *Water Sci. Technol.* **42** (12) 173-179.
McLACHLAN JA and ARNOLD SF (1996) Environmental Estrogens. The *AmericanScientist.* Accessed on 20 October 2007. http://www.amsci.org/amsci/articles/96articles/McLachla.htm.
MELNICK R, LUCIER G, WOLFE M, HALL R (2002) Workshop Summary: Summary of the National Toxicology Program's Report of the Endocrine Disruptors Low-Dose Peer Review. *Environ. Health Perspect.* **1101** (4) 427-431.
MULLER AW, WENTZEL MC, SAAYMAN GB, VAN DE MERWE SA, ESTERHUYSE CM, SNYMAN JS and EKAMA GA (2004) Full-scale implementation of external nitrification biological nutrient removal at the Daspoort Waste Water Treatment Works. *Water SA* **30** (5) 37-43.
NAUDÉ Y (2002) Extraction of persistent organic pollutants from environmental samples from selected sites: *Proc. Analytika Symposium*. Dec 2002, Stellenbosch, South Africa..
NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES (NIEHS) (2006) Endocrine disruptors. Accessed on 21 October 2007. www.niehs.nih.gov/health/topics/agents/endocrine/docs/endocrine.pdf
PORTER W, JAEGER JW and CARLSON IH (1999) Endocrine, immune and behavioural effects of aldocarb (carbamate), atrazine (triazine) and nitrate (fertilizer) mixtures at groundwater concentrations. *Toxicol. Ind. Health* **15** 133-150.
ROUTLEDGE EJ and SUMPTER JP (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environ. Toxicol. Chem.* **15** (3) 241-248.
ROZATI R, REDDY PP, REDDANNA P and MUJTABA R (2002) Role of environmental estrogens in the deterioration of male factor fertility. *Fertil. Steril.* **78** 1187-1194.
SHARPE R and SKAKKEBEAK NE (1993) Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *The Lancet* **341** 1392-1395.
SAAYMAN GB (2004) Personal communication. Operations of Wastewater Treatment Works, Daspoort Wastewater Treatment Works, Tshwane, Gauteng SA.
SWAN SH (2006) Semen quality in fertile US men in relation to geographical area and pesticide exposure. *Int. J. Andrology* **29** 62-68.
SWAN SH, BRAZIL C, DROBNIS EZ, LIU F, KRUZE RL, HATCH M, REDMON JB, WANG C and OVERSTREET JW (2003) Geographic differences in semen quality of fertile U.S. males. *Environ. Health Perspect.* **111** (4) 414-420.
TOPPARI J, LARSEN JC, CHRISTIANSEN P, GIWERCMAN A, GRANDJEAN P, GUILLETTE LJ, JEGOU B, JENSEN TK, JOUANETTE P, KEDING N, LEFFERS H, MCLACHLAN JA, MEYER O, MULLER, RAJPERT-DE MEYTS E, SCHEIKE T, SHARPE R, SUMPTER J and SKAKKEBAEK NE (1996) Male reproductive health and environmental xenoestrogens. *Environ. Health Perspect.* **104** (4) 741-776.
UNITED NATIONS ENVIRONMENTAL PROGRAMME (UNEP) (1996) Lindane, Decision Guidance Documents. Joint FAO/UNEP Programme for the Operation of Prior Informed Consent. Food and Agricultural Organisation of the United Nations. United Nations Environment Programme. Geneva.
US-EPA. VOLATILE ORGANIC COMPOUNDS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) Revision 3 METHOD 8260C (2006) US Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH.
WORLD HEALTH ORGANIZATION (2002) International Programme on Chemical Safety: Global Assessment of the State-of-the-Science of Endocrine Disruptors, WHO, Geneva.
WORLD HEALTH ORGANIZATION (2003) *Lindane. Guidelines for Drinking-Water Quality: World Health Organisation* (3rd edn.) Geneva, Switzerland.

Available on website http://www.wrc.org.za
ISSN 0378-4738 = Water SA Vol. 34 No. 3 July 2008
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