DESCRIPTIVE STUDY OF THE OESTROGENICITY OF RUN OFF WATER FROM SMALL- SIZED INDUSTRY IN THE PRETORIA WEST AREA

by

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ABSTRACT

A large number of man-made chemicals are present in the environment as pollutants and are capable of disrupting the endocrine system of animals and humans. Small-sized industry is an area where such chemicals are used and produced in abundance. There is no legislation governing the use, production and disposal of such chemicals, which studies have shown are posing a hazard to workers themselves and the surrounding communities.

Run off water from seven sites in an area in Pretoria West, with significant numbers of small-sized industries, was screened for oestrogenicity, using the Recombinant Yeast Cell Bioassay (RCBA). Chemical analyses were done for the presence of endocrine disrupting chemicals (EDCs), including p-nonylphenol (p-NP), bisphenol A (BPA), phthalate esters, polychlorinated biphenyls (PCBs) and various organochlorine pesticides, including dichlorodiphenyltrichloroethane (DDT).

The p-NP, PCBs and organochlorine pesticides were detected using a South African Bureau of Standards (SABS) in-house method: AM178 and the time of flight spectrometer, while the BPA and phthalates were detected using the CSIR Biochemtek Laboratory in-house GC-MS method: AM 186 based on the US EPA 8260 and the gas chromatography-mass spectrometer.

The water tested positive for oestrogenic activity at all the sample sites and a significant amount of lindane, an organochlorine pesticide, was detected at one site. p-NP as well as phthalate esters were identified at different sites. No pattern or relationship could be established between the oestrogenic activity and the subsequent endocrine disrupting chemicals tested for.

These EDCs in the water could pose a health risk for humans and animals. Further specific studies are needed to establish the possible sources of these contaminants, from industry and households.

Key words: endocrine system, small-sized industry, oestrogenicity, recombinant yeast cell bioassay (RCBA), endocrine disrupting chemicals (EDCs), p-nonylphenol (p-NP), polychlorinated biphenyls (PCBs), bisphenol-A (BPA), phthalate esters, organochlorine pesticides, dichlorodiphenyltrichloroethane (DDT), lindane.

OPSOMMING

Groot hoeveelheid mensgemaakte chemikalieë is teenwoordig in die omgewing en is in staat om die endokrienestelsel van diere en mense aan te tas. Klein industrieë is 'n area waar hierdie spesifieke chemikalieë in groot hoeveelhede produseer word. Daar is geen regulasies wat die gebruik, produksie, en wegdoening van sulke chemikalieë, reguleer nie. Studies het getoon dat hierdie hormoon ontwrigtende stowwe 'n gesondheidsgevaar mag inhou vir werkers.

Water vanaf sewe punte in die Pretoria Wes area, met beduidende hoeveelhede klein industrieë, is getoets vir estrogenisiteit deur gebruik te maak van die gemanipileerde gissel-estrogeentoets (RCBA). Chemiese toetse is gedoen om vir die teenwoordiheid van hormoon ontwrigting chemikalieë te toets, naamlik p-nonielfenol (p-NP), bisfenol A (BPA), phthalaatesters, poligechlorineerde bifeniele (PCBs) en verskeie organochlorien pestisiede, insluitende DDT.

Die p-NP, PCBs en organochlorien insektedoders is getoets deur die gebruik van die Suid Afrikaanse Bureau van Standaarde (SABS) in-huise metode: AM178 en die Vlugteid Massa Spectrometer. BPA en phthalates is getoets deur die gebruik van die WNNR Biochemtek in-huise GC-MS metode: AM 186 gebaseer op US EPA 8260 en die Gaschromatograafmassaspectrometer.

Die water het positief getoets vir estrogeen aktiwiteit by al die punte waarvan ñ monster geneem is. 'n Beduidende hoeveelheid lindane, is gevind by een van die toetspunte. p-NP, sowel as phthalate esters, is gevind by verskillende toetspunte. Geen patroon of verwantskap kon vasgestel word tussen die hoeveelheid estrogeen en die hormoon ontwrigting chemikalieë wat positief getoets het nie.

Die hormoonontwrigtendestowwe wat in die water gevind is, kan gesondheidsgevare inhou vir mense en diere. Spesifieke verdere studies is nodig

om te bevestig waar die hormone vandaan kom, industrieë of huisehoudelike kontaminasie.

Sleutelterme: endokrienstelsel, klein industrieë, estrogenisiteit, gemanipileerde gissel-estrogeentoets (RCBA), hormoon ontwrigting chemikalieë, p-nonielfenol (p-NP), poligechlorineerde bifeniele, bisfenol A (BPA), phthalaatesters, organochlorien pestisiede, dichlorodiphenyltrichloroethane (DDT), lindane.

DECLARATION

I declare that the dissertation that I submit is for the degree Master of Medicine (Community Health) at the University of Pretoria; it is my own work and has not previously been submitted by me for a degree at another university.

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(SI Mahomed)

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ACRONYMS

MbuP	Mono-n-butyl Phthalate
TDI	Tolerable Daily Intakes
NOAEL	No Observed Adverse Effect Level
LOAEL	Lowest Observed Adverse effect Level
ADI	Acceptable Daily Intakes
MRL	Maximum Residue Limits
DES	Diethylstilboestrol
RDP	Reconstruction and Development Programme
UP	University of Pretoria
CSIR	Council for Scientific and Industrial Research
SABS	South African Bureau of Standards
NP	Nonylphenol
SSI	Small Sized Industries
E_2	17β-estradiol
EC_{50}	50% induction of the β-galactose activity
p-NP	Para-Nonylphenol
BPA	Bisphenol A
PCB	Polychlorinated Biphenyls
RCBA	Recombinant Yeast Cell Bioassay
DBP	Di-n-butyl phthalate
DEHP	Di (2-ethylhexyl) Phthalate
BBP	Butyl Benzyl Phthalate
AR	Androgen Receptor
3,5,6-TCP	3,5,6-trichloro-2-pyridyl phosphate
EDSTAC	Endocrine Screening and Testing Advisory Committee
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
IPCS	International Programme on Chemical Safety
ATSDR	Agency for Toxic Substances and Disease Registry

GLOSSARY

Cryptorchidism	Is a condition in which one or both testicles have not descended into the scrotum.
Endometriosis	The presence of endometrial tissue in abnormal locations, is common in women of reproductive age.
Epididymis	An elongated, cordlike structure along the posterior border of the testis, whose elongated coiled duct provides for storage, transit and maturation of spermatozoa and is continuous with the ductus deferens
Epididymal Cysts	Closed epithelium-lined cavity or sac, usually containing liquid or semi-solid material within the epididymus.
Hypoplastic Testes	An incomplete or an underdeveloped testis.
Hypospadias	A developmental anomaly in which the male urethra opens on the underside of the penis or on the perineum.
Migration Limit	The amount of a chemical that is permitted to migrate into foodstuffs.
Perineum	Pelvic floor and associated structures occupying the pelvic outlet, bounded anteriorly by the pubic symphysis, laterally by the ischial tuberosities, and posteriorly by the coccyx. The region between the thighs bounded in the male by the scrotum and anus and in the female by the vulva and anus.
Phytoestrogen	Plant or naturally occurring oestrogens
Seminiferous Tubules	Involved in intense and continuous cell multiplication for spermatogenesis. Constitutes approximately 75%-90% of the total testicular mass in a mammalian adult.

Spermatogenesis	The process where spermatogonia form spermatozoa, or the production of spermatozoa.
Time to pregnancy	The number of menstrual cycles or months it takes a couple to achieve a pregnancy. This can be used as a measure of fertility.
Tolerable Daily Intakes (TDIs)	An estimate of the amount, expressed on a body weight basis, of a contaminant which can be ingested every day over a whole lifetime without appreciable health risk.
Xenoestrogens	Chemicals that mimic natural oestrogens.

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CHAPTER 1

1.1 INTRODUCTION

There is growing concern that abnormalities in male reproductive health are becoming more frequent, with rising numbers of testicular cancer cases and increases in cryptorchidism, hypospadias and decreased seminal parameters[1].

Studies have identified a class of compounds that may mimic the chemical activities of oestrogen and which are suspected of causing infertility and behavioural changes in species such as polar bears, beluga whales, alligators and humans[2].

Environmental researchers were taken by surprise in recent years by the number of "feminised" males found among several wildlife species. The effects were finally traced to pesticides and other chemicals that behave like oestrogens – but don't in any way look like them. The class of chemicals that mimic or suppress the action of hormones, particularly oestrogen, are known as endocrine disruptors [3].

Endocrine disruptors are ubiquitous, particularly in synthetic chemicals commonly used in industry. Small industries are significant users of synthetic chemicals in their production processes.

1.2. RATIONALE

In many of the developing countries of the world less than a quarter of the population have 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 are growing rapidly. This implies that the industrial, household and agricultural waste is increasing, and proper disposal of these pollutants is limited. Often, this complex mixture of toxic compounds and pollutants are disposed 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.

In this study area, the run-off water from industry and households, together with the sewage is processed via biological and physico-chemical means, before being released into receiving waters. While this treatment reduces the concentrations of viruses, bacteria and biological substances, it often does not eradicate all the chemicals, including the 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.

Measurement of human exposure to EDCs (eg. Alkylphenols, PCBs, phthalate esters, organochlorine pesticides, and BPA) is difficult and extremely expensive. There is however considerable concern, as these chemicals are ubiquitous in the environment and bioaccumulate higher up in the food chain.

1.3. RESEARCH QUESTION

Is the run off water from small-sized industry in the Pretoria West Area Oestrogenic?

1.4. OBJECTIVES

This study was conducted to:-

- Screen for oestrogenicity of run off water from small-sized industry in the Pretoria West area.
- Run chemical analysis on run off water from the Pretoria West area for specific endocrine disrupting chemicals.
- Characterize the endocrine disrupting impact of small sized industries.

1.5. LITERATURE REVIEW

1.5.1 Endocrine Disruptors

1.5.1.1 Definition

The definition according to the United States Environmental Protection Agency's (US EPA) Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) is: 'An endocrine disruptor is an exogenous substance or mixture that alters the structure or function(s) of the endocrine system (i.e. the communication system of glands, hormones and cellular receptors that control the body's internal functions) and causes adverse effects at the level of the organism, its progeny, populations or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle[4].

Environmental oestrogens, endocrine-disruptors, endocrine-modulators, ecoestrogens, environmental hormones, xenoestrogens, hormone-related toxicants, and endocrine-active compounds are all terms used to describe endocrine disrupters. These are derived from synthetic chemicals and natural plant compounds[5].

Many commonly used household and industrial substances have been implicated in causing oestrogenic effects, or general endocrine disrupting effects. Such substances include detergents, agricultural pesticides, additives to plastics, dyes, paint components and pharmaceuticals.

1.5.1.2 Oestrogens and Oestrogen mimicking Compounds

Oestrogens are steroid hormones made primarily in the female ovaries and placenta and the male testes in humans and other animals. Oestrogens are found in greater amount in females than males[6].

Oestrogen functions in the development and maintenance of secondary sex characteristics, maturation and cyclical activity of accessory sex organs, and in

general, regulates reproductive cycles (menstruation, pregnancy). It influences growth, development and behaviour (puberty) and affects many other body parts (bones, skin, arteries, the brain, etc), and thus contributes an important component of the female physiology. Oestrogen is commonly defined as "any of a family of steroid hormones that regulate and sustain female sexual development and reproductive function"[6].

In males, oestrogen is produced in the testicles and is found in sperm; however too much oestrogen inhibits the growth of the testes and production of sperm. The physiological role of oestrogens in males is unknown [6].

Estradiol is the most abundant and potent oestrogen hormone. Estrone, estriol and 17-β-oestradiol are other types of oestrogens. Oestrogens excreted from humans are usually inactive and easily dissolves in water. However bacteria in the sewage, water and sediments can convert these oestrogens into active forms (uncongugated) and result in a constant supply of oestrogens in the oceans.

In 2003, Atkinson and co workers, looked at the oestrogens in coastal marine environments and found that the highest concentrations of the natural steroid, estrone was located near sewage outfalls and underground injection wells that leach sewage into marine waters. It was suggested that the most likely source of the oestrogen was pharmaceutical products such as contraceptive pills and hormone replacement therapies. However most of this oestrogen was in an inactive form (conjugated)[5].

Various other important oestrogen mimicking compounds that are found in sewage from industry, include polychlorinated biphenyls, organochlorine pesticides, alkylphenols, and phthalate esters[5].

1.5.1.3. Phytoestrogens / Plant Oestrogens

Phytoestrogens, which are oestrogen-like compounds, are found in plants, whereas other compounds in the environment act on plants and animals with oestrogen-like effects[5].

Some chemicals that have demonstrated oestrogen activity are also found as naturally occurring substances in the environment. Examples of these are Zearalenone, which is a product of a fungus, Fusarium, which commonly infests corn[5].

Another group is the phytoestrogens, examples of which are soya, genistein, diadzein and coumestrol. Both of these have been shown to interact with the cytoplastic receptor of oestradiol. Tetrahydrocannibol (THC), one of the active components of marijuana, was also found to be oestrogenic, as there were reports of incidences of gynecomastia[7].

In laboratory tests, more than 43 plants and foods found in the human diet have been shown to be oestrogenically active and many of these phytoestrogen-containing plants are common elements in our diet, such as corn, wheat and some legumes, in particular the soya bean. The majority of soya beans are crushed for their oil. In the Unites States roughly 80% of the vegetable oil consumed is soya bean oil, and in 1999 the annual per capita consumption of soya bean oil was an estimated at 65 pounds per year. Most fried snack foods are manufactured with vegetable oil, predominantly soya bean oil. Virtually all infant formulas are a mixture of dairy and soya solids and proteins[8].

A study done in 1998 by Santti, showed that phytoestrogens from soy based feeds and isoflavoniods such as genistien given to laboratory rodents during development, produced an effect on the central nervous system (CNS) – gonadal axis and male sexual behaviour. The changes seen were similar to those seen after neonatal treatment with diethylstilbesterol (DES), but higher doses of phytoestrogens were needed[9].

1.5.1.4 Synthetic Oestrogens

Ethinylestradiol is the most common synthetic oestrogen. Some synthetic oestrogens are individually weak, but have up to 1000 times more oestrogenic activity when combined. Substances like dieldrin, toxaphene and endosulfan have individual potencies of 1/10 000th that of the natural oestrogen, 17-β-estradiol[7].

The pill is one of the best known synthetic oestrogens. It has been used for many years and has a wide variety of uses, ranging from the usage as a contraceptive agent, the alleviation of menopausal syndromes, treatment of acne, reducing menstrual irregularities in young women as well as enhancing bone resorption and preventing osteoporosis, the brittle bone disease[7]

1.5.1.5 Antiandrogens

Antiandrogens are substances that block androgen action. Androgens are steroid hormones, such as testosterone, that primarily controls male traits. They bind to androgen receptors (AR) in a cell, move into the cell's nucleus, and combine with DNA to initiate genetic transcription that leads to androgens bodily effects[5].

A study done by Chattopadhyay and team and quoted in the research briefs of 2003, shows that Bisphenol A (BPA) and Nonylphenol (NP) adversely affect the androgen receptor (AR) at many levels including blocking androgen binding, interfering with AR movement into the cell's nucleus, and disrupting genetic communication[5].

1.5.2 The Endocrine System

This is the communication system of glands, hormones and cellular receptors that control the body's internal functions. The endocrine system utilises circulating hormones to help integrate the functions of individual organs and the nervous systems. Complex interactions among these integrating systems are responsible

for control, regulation, a[illegible]omeostasis, reproduction, development, and behaviour[10].

Hormones exert their effects through binding to a specific receptor. In turn, the activated receptor initiates a cascade of biochemical events. In many cases the hormone-receptor complex interacts directly with DNA, triggering production of gene products.

Endocrine disrupting agents may act through any number of mechanisms. They may interfere with the synthesis, storage, release, secretion, transport, elimination, binding, or action of endogenous hormones. They may temporarily or permanently alter feedback loops involving the brain, pituitary, gonads, thyroid gland, or other organs. Their action is not limited to receptor binding[10].

Weak hormone agonists may, at certain doses, actually behave as hormone antagonists by blocking a receptor from occupancy by a more potent compound[10]. For example, in vivo tests have shown dioxins to compete with thyroid hormone for binding sites on transport proteins and to alter hormone production and metabolism. Dioxins may also mimic the hormone's action and block it [11].

1.5.3 Endocrine Disruptors of Importance

There are a series of industrial chemicals, some of which, like alkylphenols and phthalates, are produced in enormous quantities for multiple uses and are found throughout the world's ecosystem, and form part of the list of known endocrine disrupting chemicals[10].

1.5.3.1 Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs), are industrial chemicals now banned from production in the US, that were used as insulators in electrical equipment as heat transfer and hydraulic fluids and for a variety of other purposes, including as a coating on the inside of grain silos. PCB consists of 209 congeners, which are

found in different mixtures in commercial products. These are among the most ubiquitous and persistent environmental contaminants. These organochlorines bioconcentrate at the higher ends of the food chain, persist for decades in the environment, and are transported globally. Consumption of fatty sports fish from contaminated waters are a major source of human exposure[12].

Both oestrogenic and antiestrogenic effects have been reported for different PCB congeners. The oestrogenic potency appears to depend on the percentage of chlorine: less-chlorinated PCBs (Aroclors 1221, 1232, 1242, and 1248) have oestrogenic activity whereas the more chlorinated congeners do not. Less chlorinated compounds were shown to transfer more readily across the placenta. PCBs are hydroxylated in animals, and these hydroxybiphenyls are quite active as oestrogenic compounds, more than 1/100 of oestradiol activity[1]

The pallid sturgeon, an endangered fish and native to the Missouri and Mississippi Rivers, have shown no recent record of reproduction. All the pallid sturgeons seen today are 30 to 40 years old and high concentrations of PCBs and DDT have been found in the pallid sturgeon[7]. PCBs have been correlated with a decrease in reproductive success and thereby a decrease in populations of cormorants, gulls, herons, and other predatory birds in the Great Lakes basin[13].

PCBs do cross the placenta and expose the unborn foetus to the body burden of the mother; postnatally the breastfed baby is again exposed to PCBs via the mothers milk [14]. Experimental studies, have found PCBs to play a causative role in producing neurodevelopmental adversity in infants who were exposed to PCBs prenatally or early postnatal. Experimental evidence of hypothyroid action of PCBs is well established [14]. Studies in rats have suggested that PCB exposure affects semen quality and affects the ability of sperm to fertilise eggs. However in one of the few published human studies, it was found that infertility in males was associated with a higher semen levels of PCB than in the general population[15]. A recent study found a positive relationship between decreased human semen quality and organochlorine compounds in blood[16].

1.5.3.2 Bisphenol-A (BPA)

Bisphenol-A is an industrial chemical, used to manufacture polycarbonate and numerous plastic articles, compact disks, food can linings, thermal (fax) paper, safety helmets, bullet resistant laminate, plastic windows, car parts, adhesives, protective coatings, powder paints, polycarbonate bottles and containers (including returnable milk and water bottles and some baby bottles) and the sheathing of electrical and electronic parts. BPA is also used in PVC production and processing, where it may be used as a reaction inhibitor, and as an anti-oxidant[17].

However, recent studies have shown that it can leach out of certain products, including the plastic lining of cans used for food, polycarbonate babies' bottles and tableware, and white dental fillings and sealants into food and saliva[17].

In one study low doses of Bisphenol A has been shown to induce mammary gland growth in rats, as well as increase in prostate weight in the male offspring. However this was not a uniform finding in other studies[13].

BPA reaches the foetal brain fast, while maternal oestrogen does not enter the foetal brain. BPA has been shown to affect the brain development in rat offspring. BPA was found in numerous studies to cause behaviour in male and female rats to become similar[17].

1.5.3.3 Phthalate Esters

Phthalates are used as "plasticizers", i.e. they make plastics flexible and durable, and are commonly used in the production of polyvinyl chloride plastics (referred to as PVC or vinyl), as well as in paints, inks and adhesives. Phthalates accumulate in fat and have been found in birds' eggs, seals, fish and human fat and breast milk [5].

Soft PVC children's products are usually plasticized with phthalates. As young children suck and chew on toys, they extract and ingest certain quantities of the plasticizers. Some phthalates have been shown on animal experiments to affect the kidneys and liver and cause testicular damage[18].

Di-(2-ethylhexyl) Phthalate (DEHP), a plasticizer or softener, typically added to PVC medical products to increase flexibility, has been found to affect levels of the male hormone androgen and sperm production in male rodents as well as oestrogen levels and fertility in female rats. At high doses, DEHP was suspected to be a rodent liver carcinogen and has been replaced in many instances with diisononyl phthalate (DINP)[18].

1.5.3.4 Organochlorine Pesticides

Organochlorine pesticides include dichlorodiphenylethanes (DDT, DDD, DDE, dicofol, perthane, methoxychlor), cyclodienes (chlordane, oxychlordane, transnonachlor, heptachlor, heptachlorepoxide, aldrin, and dieldrin), hexachlorobenzene, and hexachlorocyclohexanes. Many of these, especially DDT, were used in large quantities until the 1960s, when DDT was banned or restricted in the Western countries, but is still being used in developing countries. Hexachlorobenzene, was being used in the USA until 1985[1].
Despite restrictions on their use, these compounds are still circulating in the environment because many of them bioaccumulate and become concentrated in body lipids (biomagnify)[1].

People and wildlife are exposed to many pesticides every day through food, water and their surrounding environments. The impact of DDT provides a classic example of hormone disruption at an ecosystem scale. At the top of the food chain, species including herring gulls, bald eagles, mink and peregrine falcons, have all suffered population declines from impaired reproductive systems and debilitating deformities as a result of exposure to DDT, including intersex[19].

Methoxychlor is oestrogenic in the E-screen assay as well as, in vivo in rats. Exposure of methoxychlor throughout gestation and lactation in rodents resulted in slightly smaller testes and epididymides and in lower sperm counts in male offspring than in controls[1].

Chlorinated Cyclodienes induce liver enzymes that hydroxylate testosterone. Chlordane disturbed spermatogenesis and caused dose-related damage to the testes of mice fed for 30 days with 0.08mg or 0.25mg of the active ingredient. Mating studies of dieldrin-exposed rats suggest male-dependant disturbances in fertility[1].

Hexachlorobenzene, was also reported to induce liver enzymes hydroxylating androgens. Long term studies have shown liver and kidney anomalies in exposed animals but indicate no effect on fertility[1].

Hexachlorocyclohexanes (HCHs) – Lindane, comprise several isomeric forms; these compounds are also called benzene hexachloride (BHC). Gamma-HCH has the common name lindane and is the most acutely toxic of the isomers. Lindane was reported to have both oestrogenic and antiestrogenic effects in female rats[1]. In a recent South African study, lindane was found at a concentration of 6.35ug/l in river water, in Gauteng[20]. The BHC structure is very stable and does not biodegrade easily, and as such products like lindane will be present in the environment for a long time.

1.5.3.5 Nonylphenols

Nonylphenol ethoxylates (NPEs) are a class of chemicals commonly used as "detergents" in many industrial processes and household products. In Canada, NPEs are used in the production of pulp and paper, oil, synthetic and natural textiles and leather. They are used as additives in latex paints and cosmetics, as anti-oxidants and stabilizers in some plastics and as part of the formulations in some pesticides. Common household cleaning and personal care products such

as liquid laundry detergent, all-purpose cleaners, soaps and shampoos may contain NPEs[21].

Most NPEs end up being discharged into municipal sewage systems where they break down to a highly toxic and persistent by-product, nonylphenol (NP). Sewage sludge applied to agricultural land may contain NP, and when discharged to waterways, NP can enter the food chain[21].

para-Nonylphenol (p-NP) is a degradation product of nonylphenol polyethoxylates, and has been widely used over the past 40 years. p-NP is very persistent and not readily degraded. It has a pronounced lipophilic character and it accumulates very easily in animal tissue[22].

A South African study, looking at the effect of p-NP on fertility potential in adult male rats, showed an adverse effect on weight gain, signs of epididymal toxicity as well as impaired testicular mass and sperm count at higher doses of p-NP. In this study spermatogenesis was already established at the time of exposure; p-NP still had an effect on the histology of the seminiferous tubules[23].

1.5.3.6 Synthetic Oestrogens

The diethylstilboesterol (DES) story is, of course, another tragic example of a failure to understand the consequences of human exposure to a hormonally active substance. DES was given to millions of pregnant women for over twenty years before its adverse effect on DES daughters and sons were recognised and its failure to do what it was intended to do was acknowledged[10].

In the early 1950's a double-blind placebo-controlled study on DES during pregnancy was done. This study clearly indicated that DES did not prevent spontaneous abortions. In fact, DES was associated with increases in abortions, neonatal deaths, and premature births – we now know of the wide range of other effects which become apparent after birth. When Herbst and co-workers reported the high incidence of a very rare cancer, clear cell adenocarcinoma of the vagina,

in prepubertal girls exposed to DES in utero, the U.S. Food and Drug Administration (FDA) banned the use of DES during pregnancy[1].

In Europe, approximately 200 000 French, more than 150 000 Dutch, 63 000 Czechoslovakian, and 7 000 British women were exposed to DES, whereas in the United States 4,8 million women were prescribed DES during pregnancy.

In addition, DES was used as an anabolic agent in livestock, and the general population that used dairy products and meat may have been exposed to the hormone via this route to an unknown, and probably variable, extent[1].

However, presently in the UK, despite the controversies and proven adverse health effects, DES is still being used illegally as a potent oestrogen in medicine and in feed for livestock and poultry as a growth enhancing agent, to increase the size of certain livestock for human consumption[24].

In SA, DES is being used on a very limited scale mainly to treat incontinence in dogs. It is available in a tablet form and is imported from Europe and made available to certain veterinarians only. It is not registered for use in SA and its use as a growth enhancing agent is totally banned. Chemical residues of DES in livestock that are to be slaughtered is being strictly monitored by the pharmacology laboratories in the Orange Free State (PHARMOS) and have thus found no or very small amounts of this chemical residue. According to the chief pharmacist at Onderstepoort, there are registered natural hormones such as oestrogens, progesterones and androgens that are used as growth enhancers in the meat industry and these are administered at low levels via a pellet subcutaneously. Therefore the use of DES is not needed and unlike the UK there is no illegal market for DES in SA[25].

It is clear that we are all exposed to substances with varying degrees of oestrogenic activity on a daily basis.

1.5.4 Health Effects

During the 1960s, infertility was always cited as the female's problem and not the male's. The frequency with which a male factor contributes to a couple's infertility has increased in recent years from about 10% to 25%. There has been an increased frequency of testicular cancer, and boys born with urethral abnormalities and undescended testes. Sperm counts have also declined by about a third in the past twenty years, at a rate of about 2,1% per year, and the quality of sperm has declined as well[26].

There has been a genuine decline in semen quality over the past 50 years. As male fertility is to some extent correlated with sperm count the results may reflect an overall reduction in male fertility. The biological significance of these changes is emphasised by a concomitant increase in the incidence of genitourinary abnormalities such as testicular cancer and possibly also cryptorchidism and hypospadias, suggesting a growing impact by factors that cause serious effects on male gonadal function[27].

Many substances have been associated with developmental, reproductive and other health problems in wildlife and laboratory animals. Some experts suggest that these compounds may affect humans in similar ways[24].

A birth cohort study of 577 men in Scotland over 11 years, provides direct evidence that semen quality is deteriorating, with a later year of birth being significantly associated with a reduced number of sperm in adult life. It is consistent with the hypothesis advanced by Sharpe and Skakkebaek that environmental factors acting during foetal and perinatal life can cause profound effects on subsequent adult reproduction function[28,33].

More evidence on the decline in semen quality among fertile men was shown in a follow-up study of men in Paris over 20 years. This showed a decline in the concentration and motility of sperm and in the percentage of morphologically normal spermatozoa in fertile man, independent of the age of the men. This

decline in semen quality as well as an increasing incidence of abnormalities of the male genital tract in a geographic area may have a common origin. Oestrogens or compounds with oestrogen-like activity taken by pregnant women have been suggested to affect the testicular function of male offspring adversely[29].

Meintjies and team[7], reported on a study by Wolf and team, stating that the reproductive health of women is also affected by oestrogen-mimicking substances, in the form of breast cancer. They concluded that breast cancer was four times more common amongst women who had high blood levels of DDE, a breakdown product of dichlorodiphenyltrichloroethane (DDT), than women who had low blood levels. However this was later disputed by a new study done by Krieger and team who concluded that there was no connection between pesticides and breast cancer[7].

The incidence of breast cancer in Western Europe and the USA has increased since 1940, and 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, now affect 5 million women in the USA alone. Environmental oestrogens, xenoestrogens or foreign oestrogens, have been suspected in many unexplained cases of breast cancer and the rise in endometriosis[7].

Research done by Jacobson and Jacobson in 1996 in Michigan and North Carolina, two prospective studies showed that polychlorinated biphenyls in concentrations slightly higher than those in the general population could have a long-term impact on intellectual function. In the North Carolina study infants had neurologic anomalies at birth and development delays in gross motor function during infancy, while in Michigan, deficits in foetal and postnatal growth and poorer short-term memory in infancy and at four years of age were seen. In the Michigan study, healthy mother-infant pairs were recruited from families with different consumption of Lake Michigan fish, while the North Carolina study was a general population study[14].

These findings were corroborated in laboratory animals and in prospective studies of more highly exposed Taiwanese and Japanese children born to women who consumed rice oil, contaminated with polychlorinated biphenyls and dibenzofurans[12].

The two mass poisoning events, one in Japan in 1968 (Yusho) and one in Taiwan in 1979 (Yucheng), where one to two thousand adults were accidentally exposed to high levels of PCBs through contaminated rice oil, resulted in at least five cohort studies which measured PCB-concentrations at environmental background concentrations and related these to adverse developmental, mainly neurobehavioural outcomes[14].

In a review by the World Wildlife Fund (WWF), 2000, a number of studies by various researchers, Christian and Gillies, Tisen Iguchi, Farbollini and co-workers and Aou from Japan, were quoted, on the various health effects of BPA.

Christian and Gilles from Imperial College Medical School in London have found, that bisphenol A can affect brain cells in test tube experiments. They therefore suggest that, if these substances reached the developing brain, they might be able to alter fertility and sexual behaviour. Maternal oestrogen apparently does not enter the foetal brain, but BPA has a different structure and so may behave differently[16]. Tisen Iguchi has shown that BPA can indeed very quickly reach the foetal brain, as he has injected BPA into the blood stream of a pregnant mouse on day 17 of gestation and found it in the brain of the offspring just 30 minutes later[17].

Farbollini and co-workers have shown that exposure to an environmentally relevant dose of BPA can affect brain development in offspring. They exposed female Sprague Dawley rats to 40ug/kg bw BPA from 10 days before mating until the weaning of the pups. The behaviour of the male and female offspring, exposed both in the womb and via lactation, was affected differently. However contrary to what had been expected, masculinisation of females was not observed. In males

both the motivation to explore and anxiety were reduced, while in females, the motivation to explore, and motor activity were reduced. This experiment confirms that exposure to a weak environmental oestrogen in the period of sexual differentiation of the brain can influence adult behaviour[17].

Aou from Japan has also studied the effects of BPA on behaviour and his findings suggest that exposure to low doses of BPA causes the behaviour of females and males to become similar, so that the normal differences are no longer present. However the details of this study are not publicly available[17].

A new study confirms that Vietnam veterans have an increase likelihood of getting diabetes if they have elevated levels of dioxin in their blood. Dioxin is a powerful hormone disrupting chemical found in Agent Orange, a herbicide sprayed by American forces during the Vietnam War[30].

A recent study done by Rozati and team in India revealed polychlorinated biphenyls (PCBs) in the seminal plasma of infertile men but not in controls and the concentration of phthalate esters (PEs) in infertile men being significantly higher than in the controls. The highest average PCB and PE concentrations were found in urban fish eaters, followed by rural fish eaters, urban vegetarians, and rural vegetarians. The conclusion drawn here was that PCBs and PEs may be instrumental in the deterioration of semen quality in infertile men without an obvious etiology [31].

Reproductive disorders in gastropod species, reptiles, fish, birds, and mammals have been associated with environmental factors. Several of the disorders, such as sex reversal in reptiles and vitellogenin production by male fish, may result from oestrogenic action of chemicals in the environment. Fewer data are available concerning the mammals. However some endangered species such as Florida panthers that are exposed to oestrogenic and or other endocrine disrupting contaminants show reproductive disorders comparable to those found in humans[1].

Other workers have shown that exposure to even low levels of BPA can be harmful. For example, Oehlmann and colleagues have found that BPA, at low levels can cause dramatic effects in female freshwater ramshorn snails (Marisa cornuarietis). BPA apparently stimulates egg production and causes swelling of the female sexual glands which results in blocked ducts. This blockage prevents the eggs from being transported, so that the egg containing gland is placed under extreme pressure and bursts, resulting in sterility and in some cases death due to infection[17].

A study done on adult rats by Sakaue showed that exposure of low doses of Bisphenol-A, a xenobiotic oestrogenic compound, decreased the testicular weight, and significantly reduced both daily sperm production and the efficiency of spermatogenisis. Due to the fact that Bisphenol-A decreases the efficiency of sperm production, it would suggest that it acts as an oestrogen antagonist rather than an agonist[32].

The role of exogenous oestrogens in carcinogenesis is a point to note. In 1971, an epidemiological study was published which indicated that an unusual cluster of a rare form of cancers were found in females aged between 14 to 22, all who were related to an in-utero exposure to diethylstilboestrol (DES). It is well documented that exposure of the human foetus to DES resulted in vaginal adenosis and clear cell adenocarcinoma in the female offspring. Further studies also indicate that the DES-exposed women were more likely to develop ovarian and uterine cervix cancer than those women who were not exposed. It is estimated that between 4 and 6 million Americans (mothers, daughters and sons) have been exposed to DES during pregnancy[33].

Males that were exposed to in-utero DES demonstrated a number of teratogenic effects, as well as abnormalities of the external genital tract. Their history comprised of cryptorchidism, hypoplastic testes, epididymal cysts, sperm abnormalities (decreased sperm motility, low sperm counts, and abnormal sperm forms)[33].

The dose regimen during the 1940-1950's was 10 to 12 grams of DES that was to be administered during pregnancy. This was a substantial but short term exposure which has long term sequelae[33].

1.5.5 The South African Situation

In South Africa limited research has been done in this area. Recent research by de Jager and team on the effect of p-nonylphenol an environmental toxicant with oestrogenic properties, on fertility potential in adult male rats showed that this chemical had toxic effects on both the testis and the epididymis and that both structures might be important in impairing male fertility[22].

A similar follow-up study has been done again by de Jager on the effect of p-nonylphenol on the fertility potential of male rats after gestational, lactational and direct exposure. This study emphasised the toxicity of p-nonylphenol on both the testis and epididymis and thereby having an indirect effect of male fertility in rats[23].

A further study done by de Jager and others on the additive effects of p-Nonylphenol and phytoestrogens on reproductive parameters in male rats showed a general trend in decreased fertility parameters in the phytoestrogen and p-NP combined group. These results support the concerns that phytoestrogens in combination with other endocrine disrupting chemicals have an additive effect, resulting in decreased fertility potential[34].

Meintjies and team, wrote an extensive report for the South African Water Research Commission on the Qualitative and Quantitative Evaluation of Oestrogen and Oestrogen-Mimicking Substances in the Water Environment. The report identified those compounds that are most likely to occur in the South African situation, evaluated the oestrogenic substances that are economically important and analytically feasible for analysis and most importantly identified specific chemicals which have been claimed to mimic oestrogens[7].

The above report was followed by a pilot study by de Jager, looking at the oestrogenic contamination of South African river waters. Rivers in Mpumalanga, Gauteng and North West tested positive for oestrogenic activity. Surprisingly Pretoria drinking water also contained an oestrogenic chemical p-NP[20].

There is some relevant research done by van Wyk and team, from Stellenbosch University, following on several of the South African Water Research Commission (WRC) funded projects. Van Wyk and team have done work on oestrogenic activity in water, in 2002, by looking at the yolk precursor protein, Vitellogenin (Vtg), as a test for oestrogenicity. Polyclonal and recently monoclonal anti-Xenopus Vtg antibodies were developed and employed in a sensitive Xenopus Vtg ELISA system. Using seabream anti-Vtg antibodies, a Tilapia Vtg ELISA system was also established. Recently, a universal Vtg ELISA, to be used for screening Vtg in plasma of most vertebrate species without the use of species specific anti-Vtg antibodies, was developed and validated. These bioassays were specifically set up to asses the oestrogenicity in the natural water resources in the Western Cape agricultural region and these projects have been extended for further development, validation and implementation of bioassays to include assessments of androgenic, thyroid and immune system disruption[35].

The international launching of the USA-EPA report based on the recommendations of the Endocrine Screening and Testing Advisory Committee (EDSTAC), initiated an effort within the WRC to develop a research framework on EDCs in water in South Africa. This framework focussed on creating the capacity within South Africa to develop and validate biomarkers associated with endemic wildlife species, and also to create expertise and to facilitate development and establishment of techniques and technology to facilitate chemical analyses of water samples, with emphasis on EDCs in water resources in SA, with specific areas of concern, such as agricultural areas, industrial effluent, and drinking water[35].

1.5.6 Small-sized Industry and Importance of Research in this Area

Over the last few decades, small-sized industries (SSI's) have increasingly assumed a more important role in the social and economic life of South Africa. Latest figures have shown that small sized industries (SSI's) contribute 35% of the national GDP and employ half of the formally employed South African workforce[36].

Despite its vital role in the economy, there is increasing concern from health workers and worker organisations that SSI's may become a significant source of health concerns, both for workers employed in this sector as well as by communities surrounding SSI's. The concerns stem from the lack of adequate legislative controls to protect workers and the environment.

Some of the problems faced by these sectors are, lack of commitment by management to occupational health and safety, lack of occupational services, lack of reporting of occupational injuries, diseases, as well as incidence rates, and lack of unionisation[37].

One such occupationally and environmentally related hazard which workers are exposed to in these small-sized industry, is oestrogenically active compounds, like alkylphenol and related compounds which are products from the widely-used industrial surfactants used in detergents, paints, herbicides, cosmetics, as anti-oxidants, lubricants, plasticizers etc.

Current legislation does not demand documentation on oestrogenicity before it can be used in foods or commercial products. Man and wildlife are exposed to a very wide range of chemicals and for the majority we do not know whether these are estrogenic, whether their effects are additive or what the true exposure to the chemicals is.

In South Africa we find that the larger organizations or industries have adequate and mostly excellent occupational and environmental health services. Frequently, however, the general health of the worker is not accorded the necessary attention

and seldom are the health needs of the worker's family given serious consideration. This obviously applies to an even greater extend to small undertakings and self-employed persons and especially to agriculture[38].

Legislation is often inadequate, or totally absent for this sector. The Occupational Health and Safety Act of 1993 clearly defines legislation for industries/ organisations employing 50 or more workers, but no legislation for smaller industries or organizations is stated. Often the occupational health services (OHS) provides mainly a curative service, because it is easy to define, with high customer satisfaction indexes[37].

The characteristics of the workforce may also contribute to increased risk of occupational and environmental ill health among workers employed in Small-Sized Industries (SSI). SSI workers in developing countries are often young, poorly educated, unskilled and inexperienced. Their often limited training may result in many of them being ignorant of workplace hazards, safe work practices as well as appropriate preventive measures to take while working. This relative lack of health and safety consciousness often results in greater exposure to toxic substances. This is further aggravated by the generally longer hours of work for these workers, which often exceed the 8-hour workday or 40-hour working week for employees in large companies[40].

In many cases the workers do not demand OHS because of ignorance of the hazards they are exposed to, fear of antagonizing management, and ignorance of what OHS can offer, and ignorance of their rights. The current high unemployment rate also contributes to the fear of bringing health concerns to the fore, by the employees[37].

Health care authorities are familiar with and accept as routine, the need to provide health care for certain sectors of the community, such as mother and child, the aged, the handicapped, and the school child; but the working population is often not formally recognized and accorded this facility[40].

The working population may be exposed to health hazards over and above the rest of the community because of work practices, thereby providing another reason for special concern for their health.

The Ministry of Labour produced a 5-year Programme of Action in which it claims that existing laws regulating occupational health and safety do not comply adequately with the RDP. It also alleges that South Africa does not have an overall national policy or strategy on occupational and environmental health and safety and states that there is no consistent legislative structure, no uniform method of reporting accidents and disease, and no statistics that reflect the full extend of the loss of life and health problems caused at the workplace[41].

It is reported that there are two government agencies that deal with occupational health and safety, namely:

- The health and safety inspectorate responsible for the enforcement of health and safety standards and the prevention of occupational accidents and diseases located at different government departments (labour, health, minerals and energy, agriculture and to a lesser degree environmental affairs).
- The compensation agencies are located in separate departments (health and labour) which are responsible for compensating workers who contract occupational diseases / injuries, and the families of workers who are killed as a result.

A third separate department deals with environmental health, safety and conservation, namely the department of Environmental Affairs and Tourism.

The most severe consequences of this division of responsibility are the occupational and environmental health problems, which are under-regulated and under-reported. Achieving the ideals of the Reconstruction and Development Programme (RDP) will require a reconsideration of occupational health and safety

legislation and the structure and policies of the government agencies responsible for enforcing the legislation and setting of safety standards[42].

The Ministry of labour intends developing an overall national policy and strategy on occupational health and safety. It intends to create a National Occupational Health and Safety Council[42].

How concerned should we be about the potential abnormalities in the reproductive health of male workers employed in SSI's, particularly those that use chemicals of unknown composition. Furthermore, what potential impact do these industries have for communities whose water suppliers may be contaminated by run-off water from these industries?

These two concerns lie at the heart of this study. As a first step to providing answers to these concerns, it was necessary to first establish whether endocrine disruptors were evident and if they were, what agents were present and at what levels.

CHAPTER 2

2.1. STUDY DESIGN

This is a descriptive study involving the testing of run-off water for oestrogenicity using specific tests and chemical analyses for specific endocrine disrupting chemicals. This study attempts to test run-off water at one point in time.

The research was conducted in the Pretoria West area, Gauteng Province, South Africa. The area in question is approximately 2,6km x 1,8km, 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 contains the biggest load of small-sized industries in this area.

Figure 1: Map of Sample Area

2.2 SAMPLE SELECTION

The research was done in two phases. Phase one involved the identification of the various different types of industries in this area and the use of industrial detergents and other cleaning agents in the various processes. Attached is a list of industries and the types of detergents being used (Appendix 1).

2.2.1 Pilot Study

The first phase enumeration study, covered 169 small-sized industries in a specified demarcated area in the Pretoria West area, where the majority of the small-sized industries are located. All industries in the study area employ less than 50 workers. These industries comprise mainly food premises, panel beaters, auto body repair works, auto-spray painting services and several general dealers. Of the 169 industries, there were at least 17 industries that did not complete the questionnaire administered. Attached is the questionnaire administered (Appendix 3). The high-risk industries were 49. These have been identified as high risk on the basis of their use of industrial detergents.

2.2.2 Phase Two

In phase two, run-off water was collected from 7 different points in the study area (see Table 1)

The selection of test points was done in consultation with city engineers from the Water and Consumer Management Division of the Tshwane Department of Water and Sanitation. The criteria used for the selection were points where water converges from the high-risk industries that were identified in phase one of the project. The first five points represent points of convergence in the test area. These are the most northerly points, i.e. the water moves in a northerly direction towards the water works. The sixth was at the entrance of the Daspoort Water Works and the final one was at the exit of the water works, just as it enters the Apies River.

Water samples were sent for screening for oestrogenicity and for chemical analyses. The screening test done, was the Recombinant Yeast Screen Bioassay. In addition, the water samples were sent for a chemical analysis which was used to detect PCBs, BPA, p-NP, phthalate esters [DEHP di-(2-ethylhexyl)-phthalate, DBP di-n-butylphthalate, BBP butylbenzylphthalate], and the Organochlorine pesticides.

Table 1 – Run-off water collection points

Sample Collection Point	Location
1	Corner of Buitekant and Church Street
2	Corner of Rebecca and Church Street
3	Corner of Zieler and Church Street
4	Corner of Zieler and Church Street (opposite end of collection point 3)
5	Corner of President Burger and Church Street
6	At the entrance of Daspoort Wastewater Treatment Works
7	At the entrance of the Apies River

2.3 QUALITY ASSURANCE

2.3.1 Training

The city engineers from the Tshwane Water and Sanitation Department are professionals who are qualified in Water Pollution Control.

The technicians at the Andrology Laboratories, UP as well as at the Chemical Laboratories of SABS and CSIR have qualified technicians who are running similar tests for many years and are fully qualified to carry out these tests and document results.

2.3.2 Monitoring

At the collection points, I collected the water personally and was assisted by the City Engineers.

Each glass bottle was covered with aluminium foil and then capped with a plastic bottle top to prevent leaching of oestrogens into the water.

Once the water samples were collected and stored in the pre-treated bottles provided by the Andrology laboratories, they were transported directly to the laboratory by me personally where they were stored under optimum conditions in a refrigerator at 5°C.

The various supervisors, at the different laboratories constantly monitored the work of the laboratory technicians as well as assisted the technicians with the various screening and biochemical tests.

2.3.3. Data Entry

The tests were done over a two week period. The results were read daily by the laboratory technicians for a period of 10 days and entered into a database. These results were looked at collectively and analysed using the STATA computer package by the technician. A statistician from the SHSPH evaluated the results.

Further, each screening test at the Andrology laboratory is controlled on its own, thereby ensuring further quality control measures.

2.4 SAMPLING TECHNIQUES

The water samples were collected in two-litre glass bottles provided by the Andrology Department of the University of Pretoria. 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. This prevents the plastic top from leaching oestrogens into the water, thereby contaminating the sample.

At each sample point, a stainless-steel beaker was suspended by a rope and lowered into the specific selected man-hole and water was collected into it and poured into a marked bottle. The beaker was rinsed with ethanol before being lowered at each site. This prevented cross-contamination of water. Despite the fact that there were areas where the level of the water was low and other areas where the flow of water was too fast, in each case, an adequate sample was obtained.

These water samples were collected with the assistance of engineers from Water Consumer Management of the Tshwane Department of Water and Sanitation. The specific sites were accessed using a motor-vehicle and the man-hole lids were opened with a pick-axe, photographs below (Figs 2,3,4,5,6 and 7).

The samples were collected 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 and their plants or sites.

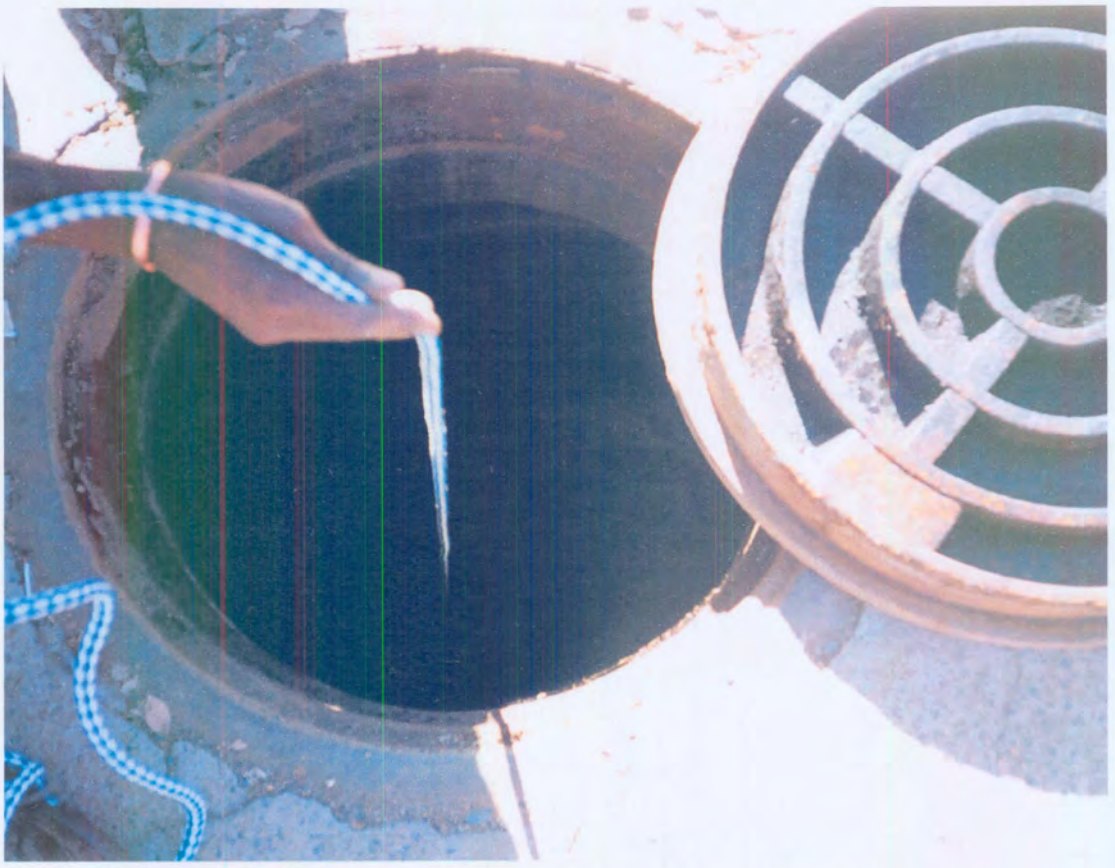


Figure 2: Sample 2, Corner of Rebecca and Church Streets

Figure 3: Sample 3, Corner of Zieler and Church Street

Figure 4: Sample 5, Corner of President Burger and Church Street

Figure 5: Daspoort Wastewater Treatment Works

Figure 6: Sample 6, Entrance of Daspoort Sewage Treatment Plant

Figure 7: Sample 7, Entrance of Apies River (Exit of Daspoort Wastewater Treatment Works)

2.5 ANALYTICAL METHODS

Methods used to determine oestrogenicity are in general expensive and time-consuming. A variety of short-term assays are therefore applied to identify oestrogenic chemicals and to determine the relative potencies for hormonal responses. Some of these assays may be suitable for screening large numbers of chemicals and contaminated media, such as water and food, and may therefore be useful tools for prioritising chemicals for more extensive studies in vitro. In this study one method was used to determine oestrogenicity – the Recombinant Yeast Cell Bioassay (RCBA).

Chemical methods were used to detect p-Nonyl phenol (p-NP), Polychlorinated Biphenyls(PCB) and Organochlorine Pesticides and these were carried out by the SABS laboratories, Phthalate Esters (DEHP, DBP and BBP) and Bisphenol-A (BPA) were detected chemically by the CSIR Bio-Chemtek laboratories.

Two laboratories were used to analyse the endocrine disrupting chemicals as all the chemical analyses could not be done by the SABS laboratories alone as was initially planned. The SABS laboratories do not have the standards needed to carry out the analyses for phthalates and BPA, nor do they have a Gas Chromatography-Mass Spectrometer to do the final analyses on the phthalates and BPA.

2.5.1 Recombinant Yeast Cell Bioassay (RCBA)

The extracts of the products used for the GC/MS detection's were dissolved in ethanol and used to test for oestrogenicity.

The particular advantage of the transformed yeast cell line approach is that the screening assay reflects the potential oestrogenic activity in humans. The yeast screen test is at present regarded as the most sensitive test available for oestrogenicity for environmental samples. (The RCBA is twice as sensitive as compared to the MCF-7 cells and five times more sensitive than the uterotrophic

assay)[43]. The oestrogenic potency is compared to that of 17ß-estradiol (E2), a natural oestrogen used as a positive control in in-vitro assays.

The endpoint measured in this assay is ß-galactose activity. Calibration curves of E2 and water compounds are constructed by analysis of standards in selective media for oestrogenic activity. Each calibration standard of a test compound was analysed in 8 wells (1 column) and the assay blanks (vehicle only) in 16 wells (2 columns) of a 96-well microtitre plate. Each test compound was assayed in a separate 96-well microtitre plate and the wells were sealed with film plate sealers.

ß-Galactosidase activity in blank wells were subtracted from wells containing standard concentrations of the test chemicals. The relative potency of test compounds were determined from the concentration of E2 and test compound that provided 50% induction of ß-galactose activity (EC_{50}) and calculated by dividing the E_2 EC_{50} by the test compound EC_{50} x 100[44].

2.5.1.1 Sample preparation

The assay was carried out in a type II laminar flow air cabinet, to minimise aerosol formation. Serial dilutions were made of the water sample extracts and controls, in 96-well microtiter plates (Cat. No. 95029780, Labsystems). 100µl of the solvent, ethanol (Cat. No. 27,0741, Sigma-Aldrich) was placed in wells 2-12 on the plate. 200µl of the sample extract was placed into the first well and this was serially diluted (100µl) across the plate, using a Finpipette multichannel pipette (AEC Amersham). 10µl aliquots were then transferred to a 96-well, optically flat bottom microplate (Cat. No. 95029780, Labsystems). This was allowed to evaporate to dryness on the assay plate.

Aliquots (200µl) of the assay medium containing the yeast and chromogenic substrate (CPRG) were then dispensed into each sample well using a multi-channel Finnpipette. Each plate contained at least one row of blanks (assay medium and solvent ethanol) and a standard curve for 17β-estradiol (Cat. No.

E8875, Sigma) ranging from $1x10^{-8}$ M to 4.8×10^{-12} M (2.274µg/l to 1.3ng/l) which was extended to a concentration of 1.19×10^{-15} M (3.24×10^{-13} g/l). The plates were sealed with parafilm (Cat. No. P7793, Sigma) and placed in a naturally ventilated incubator (Heraeus, B290) at 32°C for 3 to 6 days.

After 3 days incubation the colour development of the medium was checked periodically at an absorbance (abs) of 540nm for colour change and 620nm for turbidity of the yeast culture (see figure 9). The absorbance was measured on a Titertek Multiskan MCC/340 plate reader to obtain data with the best contrast. After incubation the control wells appeared light orange in colour, due to background expression of β-galactosidase and turbid due to the growth of the yeast. Positive wells were indicated by a deep red colour accompanied by yeast growth. Clear wells, containing no growth indicated lysis of the cells and colour varied. All experiments were performed in duplicate.

The following equation was applied to correct for turbidity:

> Corrected value = test abs (540nm) - [test abs (650nm) - median blank abs (620nm)]

The 17 β-estradiol standard curve was fitted (sigmoïdal function, variable slope) using a Graphpad Prism (version 2.01), which calculated the minimum, maximum, slope, EC_{50} value and 95% confidence limits. The detection limit of the yeast assay was calculated as absorbance elicited by the solvent control (blank) plus three times the standard deviation. The estradiol equivalents of the water samples were determined by calculating the 50% effect of the sample [(max abs – min abs)/2) + min abs]. This absorbance was then interpolated from the oestradiol standard curve and corrected with the appropriate dilution factor for each sample[43,44].

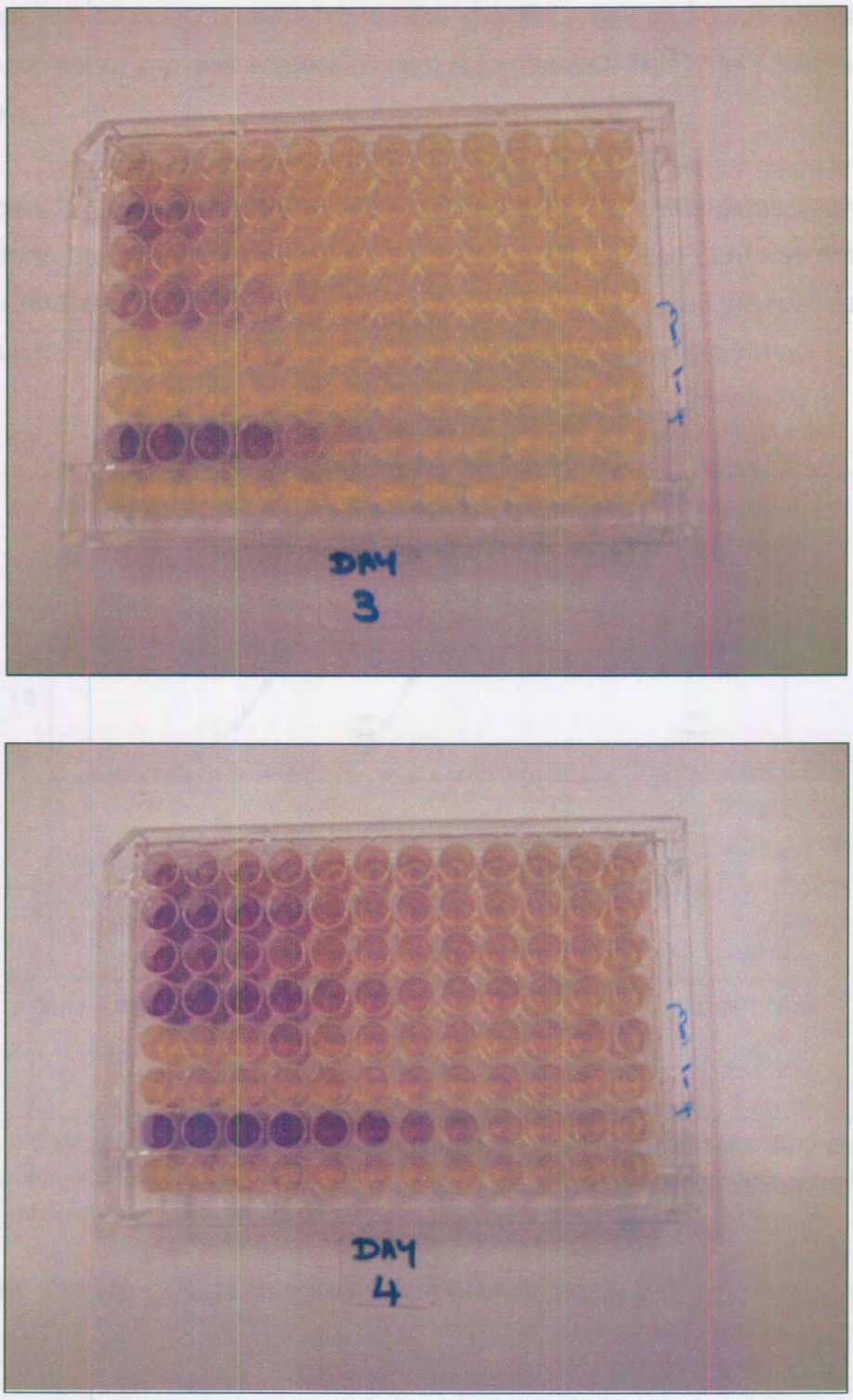


Figure 9: Example of a plate after incubation for 3 and 4 days

2.5.2 Chemical Analysis

2.5.2.1 p-Nonyl phenol (p-NP), Polychlorinated Biphenyls (PCB) and Organochlorine Pesticides (SABS Labs)

To test for p-NP, PCB and Organochlorine pesticide residues in water samples, the SABS laboratory used single determinations, a SABS in-house method: AM178 A. This is a multi-residue method to detect organochlorine, organophosphorus, pyrethroid pesticides, nonyl phenol and polychlorinated biphenyls in water.

A recovery determination was carried out by adding a known amount of pollutants to distilled water and analysing it concurrently with the samples.

The extraction and analysis of persistent organic pollutants in water were carried out using the following method: 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 cartridge was dried and the analytes were eluted with a mixture of acetonitrile and hexane, followed by hexane. The extract was evaporated to just dry at 35^0C using a rotary evaporator. The residue was reconstituted with 1ml hexane and vortexed before it was transferred to a vial.

One micro-litre of the extracts were injected onto a Agilent 6890 GC equipped with a Agilent autosampler and a LECO Pegasus II MSTOF (Time of Flight Mass Spectrometer).

The limits of detection (LODs) for Organochlorine Pesticides and PCBs was 0.1ug/L and NP was 1ug/L [45].

2.5.2.2 Phthalate Esters (DEHP, DBP and BBP) (CSIR Labs)

The method used by the CSIR laboratories was the liquid-liquid extraction of the sample using dichloromethane as an extraction solvent. The final analysis in this case is by Gas Chromatography of Gas Chromatography-Mass Spectrometry (see figure 10).

The above phthalates were determined using an in-house GC-MS method AM 186 based on US EPA 8260. The lowest limits of detection using this method are:

DEHP - 4ug/litre
DBP - 3ug/litre
BBP - 4ug/litre

This method is used to determine the concentration of semi-volatile organic compounds in extracts prepared from environmental samples. This is a target compound analysis that includes neutral, acidic, and basic organic compounds that are soluble in methylene chloride.

An aliquot of sample was taken, surrogate was added, and the pH adjusted to 2 with sulphuric acid and extracted using methylene chloride. The pH of this solution was adjusted to 11 using sodium hydroxide and was extracted again using methylene chloride. The sample extract was then concentrated to 1 ml. Internal standard was added to the sample prior to injection on the GC-MS.

The sample is introduced to the GC-MS by injecting the extract into a gas chromatograph with a narrow bore fused-silica capillary column. The GC column is temperature programmed to separate the analytes, which are then detected with a mass spectrometer (MS) interfaced to the gas chromatograph (GC). Analytes eluted from the capillary column are introduced into the mass spectrometer via a direct connection. Identification of the target analytes is accomplished by comparing their mass spectra with the electron impact spectra of authentic standards. Quantification of each component is accomplished by comparing the

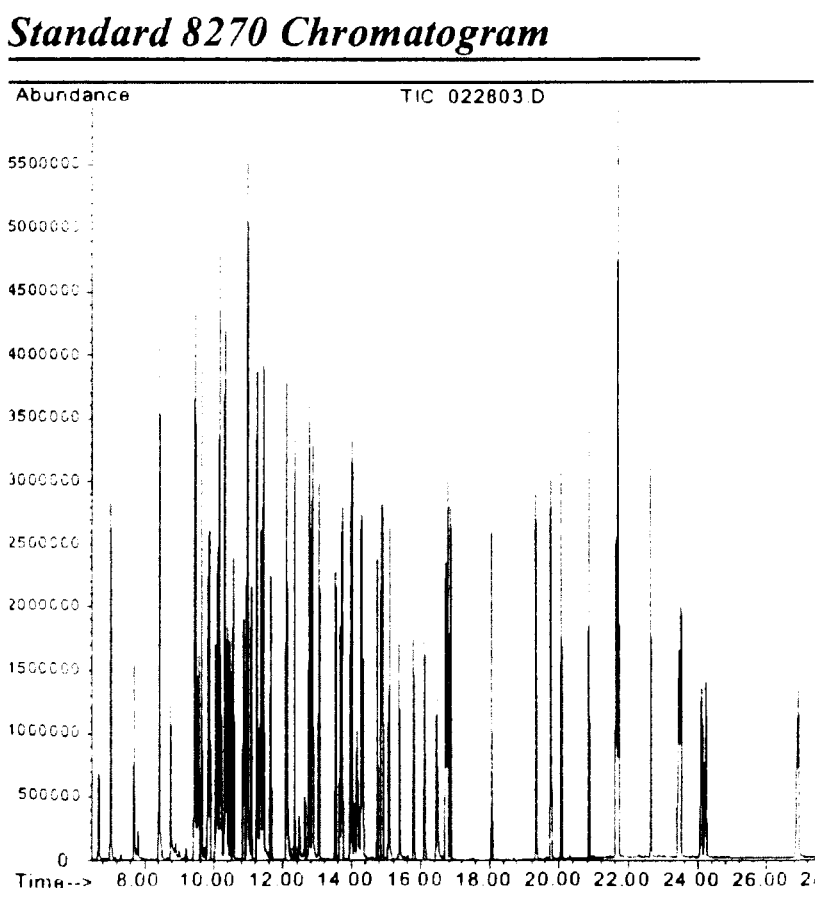


Figure 10: An Example of a Diagram showing a Standard Chromatogram

2.5.2.3 Bisphenol A

An aliquot of the sample was taken, surrogate added, the pH adjusted to 2 with sulphuric acid and extracted using methylene chloride. The pH of this solution was adjusted to 11 using sodium hydroxide and was extracted again using methylene chloride. The sample extract was then concentrated to 1 ml. Internal standard was added to the sample prior to injection on the GC-MS.

Although the same analytical procedure was followed, bisphenol-A is not a target analyte for EPA 8270. A separate calibration curve was constructed, and the same quantification procedure given above was followed [46].

Although the same analytical procedure was followed, bisphenol-A is not a target analyte for EPA 8270. A separate calibration curve was constructed, and the same quantification procedure given above was followed [46].

CHAPTER 3

3.1 RESULTS

3.1.1 Recombinant Yeast Cell Bioassay (RCBA) – Oestrogenic Activity

The results of the RCBA for each of the sample points is summarised in Table 2 below.

Sample number	Estradiol equivalents (g/L)*
1	Toxicity detected
2	9.48×10^{-13}
3	8.16×10^{-13}
4	5.8×10^{-9}
5	1.28×10^{-11}
6	2.44×10^{-9}
7	2.37×10^{-11}

* Based on the EC_{50} value of the dose response curves obtained for 17ß-estradiol (E_2) and the test samples.

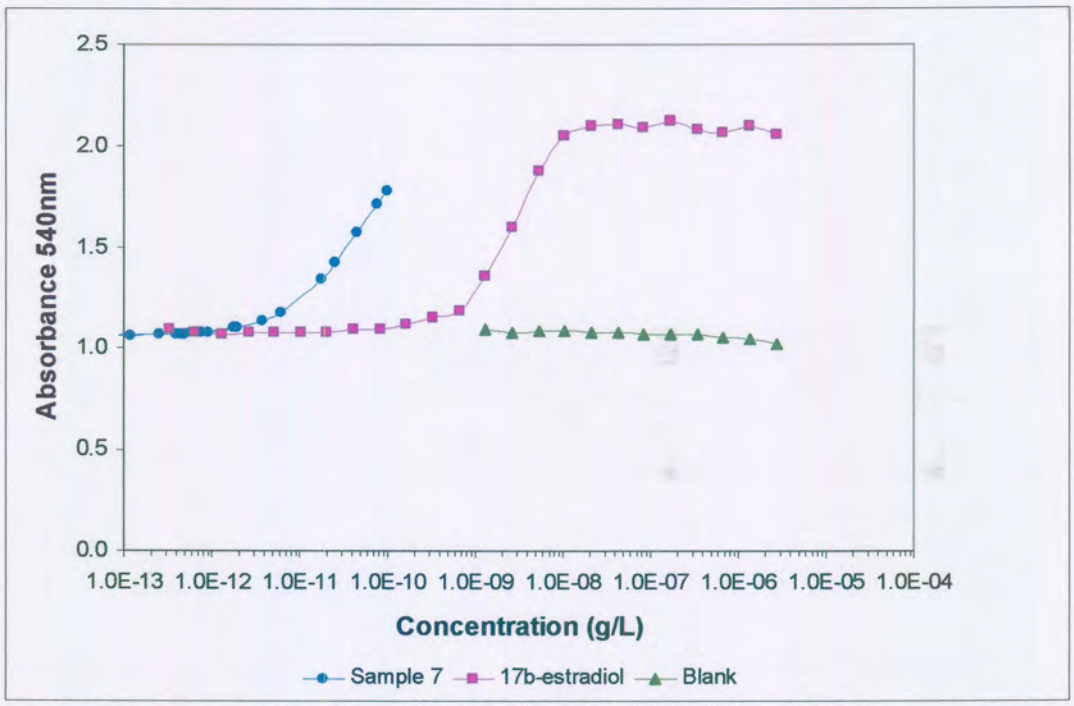
3.1.1.1 Comments and explanatory notes on the results of the RCBA

Oestrogenic activity was not detected in Sample 1 due to extreme toxicity. A toxic response implies an absorbance of the sample below the detection limit of the assay. For example, if the absorbance of the blank is 0.800 and the detection limit for the assay is 0.815, then absorbance would be negative as any absorbance below the detection limit is regarded as negative. This is usually seen by no colour change (remains yellow), no background expression of β-Glycosidase and no turbidity because the yeast cells have been lysed.

In this assay there were 24 serial dilutions resulting in a fairly low level of dilution.

Lowest limit of detection depends on the specific assay and curve for that assay. For each set of samples a specific curve is formulated. This differs from sample to sample.

Samples 3,4, 5, and 6 also showed slight toxicity, the oestrogenic activity might therefore be underestimated in the assay. This implies that on the curves formulated, the initial values were below the detection limit and the rest were along the curve. Figure 11 represents the curve formulated for sample 7



NOTE: Log concentration of 17-β estradiol serially diluted from 2.72 x 10-6 g/l to 3.24 x 10-13 g/l and the log concentration of the sample 7, after the dilution factor has been calculated plotted against the absorbance (540nm) of the medium after 4 days incubation.

Figure 11: Oestrogenic activity for sample point 7

3.1.2 From South African Bureau of Standards

3.1.2.1 PCBs, p-Nonyl phenol and Organochlorine pesticides

Table 3 summarises the results of the three chemical analysis performed by the SABS labs for each of the seven collection points.

Table 3: Summary of Results, Chemical Analysis performed by the SABS

Sample point	Organochlorine pesticides (µg/L)	Polychlorinated biphenyls (µg/L)	p-Nonyl Phenol (µg/L)
1	Not detected	Not detected	**119**[1]
2	Not detected	Not detected	Not detected
3	Not detected	Not detected	**10**[1]
4	Not detected	Not detected	Not detected
5	Not detected	Not detected	**20**[1]
6	Not detected	Not detected	Not detected
7	**Lindane = 0.9**	Not detected	**10**[1]

NOTES:

1 - Values are corrected for a recovery of ten percent for p-Nonyl phenol

According to the European Union (EU) standard for drinking and ground water, the pesticide level must not exceed 0.1µg/L for individual compounds and some of their degradation products; and the sum total of all pesticides should not exceed 0.5µg/L.

3.1.2.2 Pesticides detected but not quantified

Samples 3 and 7 showed traces of 3 organochlorine pesticides that were not part of the list of pesticides that were tested for. These pesticides are listed below

Sample 3 - Terbutylazine

Sample 7 - Chlorpyrifos and Atrazine

3.1.2.3 Recovery Determinations

Recovery determinations for all three chemical analysis performed by the SABS yielded the values listed in Table 4.

Table 4: Limit of determination and recovery range

Analyte	Method No.	Technique used	Limit of determination	Recovery range, %
Organochlorine pesticides	In-house method AM178A	GC-MSTOF	0.1µg/L	56-138
Polychlorinated bihenyls		GC-MSTOF	0.1 µg/L	52-130
p-Nonyl Phenol		GC-MSTOF	1 µg/L	10

EXPLANATION OF LIMIT OF DETERMINATION

A known concentration of p-NP was added to the sample in order to establish the extraction efficiency, only 10% of the original p-NP that was added to the sample was recovered and the results were adapted accordingly. The method is suited to water samples only. These samples had a high organic matter content and had to be filtered. p-NP is associated with the organic matter content, and thus part of the p-NP present was filtered out. For these reasons the results were corrected for recovery in order to represent the concentrations present in these samples.

Recovery determinations gave the following values. For each batch of samples, control samples were spiked by adding known quantities of authentic reference materials to the samples at the following concentrations: OCs and PCBs 0.1µg/L and NP 1µg/L. This spiked sample is worked up following the same method as for the test samples in order to determine the extraction efficiency: For example if 1µg/L analyte is added and 0.8µg/L, is recovered, then 80% of the analyte has been recovered.

Limit of determination (LOD) is the lowest detectable quantity or the lowest limit of detection and in this case the LOD is defined as the concentration that the control sample was spiked with.

Recovery range is between of 56-138%.

3.1.3 From Council for Scientific and Industrial Research

3.1.3.1 Phthlate Esters and Bisphenol-A

The results of the chemical tests conducted by the CSIR are summarised in Table 5.

Table 5: Summary of Results, Biochemical Analysis performed by the CSIR

Sample Point	DEHP (µg/l)	DBP (µg/l)	BBP (µg/l)	Bisphenol-A* (µg/l)
1	35	7	-	-
2	15	-	-	-
3	5	4	-	-
4	41	5	-	-
5	47	-	-	-
6	69	11	-	-
7	5	-	-	-

NOTE:
MDL is the lowest limit of detection for the various compounds using the in-house GC-MS method AM 186 based on US EPA 8260. Minimum Detection Levels (MDL) were as follows:- DEHP (4µg/L), DBP (3µg/L), BBP (4µg/L), and Bisphenol-A (50µg/L)

Levels for DEHP range from a low of 5 (for samples 3 and 7) and a high of 69 for sample 6. Sample 6 also demonstrated the highest level for DBP. In none of the samples were BBP or Bisphenol–A detected using the above method.

3.1.4 Summary of all the Results

A summary of the results of the various screening tests and biochemical analysis for each sample point is summarised in Table 6.

Table 6: Summary of results for each sample point

Sample point	Oestrogenicity E2 Level g/L	p-NP µg/L	DEHP µg/L	DBP µg/L	Organochlorine Pesticides µg/L
1	Toxicity detected	119	35	7	-
2	9.48×10^{-9}	-	15	-	-
3	8.16×10^{-13}	10	5	4	Terbutylazine (not quatified)
4	5.8×10^{-9}	-	41	5	-
5	1.28×10^{-11}	20	47	-	-
6	2.44×10^{-9}	-	69	11	-
7	2.37×10^{-11}	10	5	-	Lindane - 0.9 Atrazine (not quantified) Chlorpyrifos (not quantified)

3.1.5 Limitations to the Study

There were several limitations with this study. These include:-

- This is a descriptive study and it cannot prove or disprove any links or relationships between exposure and outcomes

- The Recombinant Yeast Screen Bioassay can only comment on oestrogenicity of the water samples. This test cannot identify specific chemicals.

- These screening tests if positive will not be able to identify the source of the oestrogenicity, ie the type of detergent, if at all it was a detergent that is responsible for the oestrogenicity of the sample.

- In vitro assays do not always reliably predict the outcome of in vivo assays, since chemicals can be metabolically activated or inactivated in vivo, and may act independently of the receptor[47].

- The RCBA was the only oestrogenic screening test done in this study. The other two tests; MVLN and E-Screening tests which were planned for could not be done as the laboratories, had problems with contamination and setting up the MVLN system. Thus the sensitivity of the RCBA could not be confirmed.

- The inconsistent results between different in vitro systems may also be partially due to the differing metabolic capabilities of the test systems used. Thus, whether a chemical is, or is not, identified as being oestrogenic may depend on the actual test system used, and this calls for confirmation of any positive findings using other assays[47].

3.1.6 Data Analysis

The statistical analysis was performed with the assistance of, a statistician from the School of Health Systems and Public Health, University of Pretoria.

The Wilcox's Signed Rank Test, which is a non-parametric test was used to compare the two methods with each other.

The statistical package, STATA version 7 was used for data entry and analysis.

CHAPTER 4

4.1 DISCUSSION

Sample 1 demonstrated toxic levels of oestrogen, with corresponding high levels of p-NP, DEHP and DBP, but no organochlorine pesticides. Sample 1 was taken from the man-hole at the corner of Buitekant and Church Street in the Pretoria West area. This is an area with dense small industries, namely motor service and repair shops, a petrol station and several food outlets.

Sample 2 also demonstrated oestrogenicity, and only a positive DEHP level. This sample was taken at the corner of Church and Rebecca Street. This area has mainly small food outlets.

Sample 3 showed signs of slight toxicity as far as the oestrogenicity goes, with corresponding positive findings for p-NP, DEHP, DBP and for terbutylazine, an organochlorine pesticide that was not quantified.

Sample 4 was positive for oestrogen, DEHP and DBP.

Samples 3 and 4 were taken from the corner of Zieler and Church street on either sides of the street. 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.

Sample 5 showed oestrogenicity, p-NP as well as DEHP. This sample was taken from the corner of President Burger and Church Street, which is a slightly less dense area than the rest. There are a few households and some light industries in that area.

Sample 6 showed oestrogenicity, but no p-NP was detected, however high amounts of DEHP and DBP were present. There were no organochlorines present in this sample. This sampling point is, where all the water from the greater Pretoria West area converges, before it enters the Daspoort Sewage Treatment Plant. One would expect to find organochlorine pesticides here; at this point effluent converges from all the industries, and from the few households in this area.

All water; effluent from industry and households as well as sewage drains into the same drainage system, and converges to the Daspoort Wastewater Treatment Works, where it is treated, and thereafter dispelled into the Apies river. The storm water, however drains into a totally separate system. Storm water drains empty out into the river directly. Storm water should not at any point be contaminated with industrial effluent, this is legislated and monitored strictly by the water and sanitation department.

Sample 7, which is the last sample, was taken from water that had just passed through the Daspoort Wastewater Treatment Works and is then discharged into the Apies River (see Fig 7). This sample has positive oestrogenic activity, detected at a level of $2.37x10^{-11}$, and some p-NP and DEHP present. There are organochlorine pesticides, viz lindane, atrazine and chlorpyrifos, which were present in sample 7 and not in sample 6. There are some discrepancies between sample 6 and 7, which needs further testing and investigation.

The treatment process at Daspoort involves, initial screening for bigger solids, such as rags, paper, etc., thereafter the water flows through a grit channel or a grit tank to remove all solids with a specific gravity greater than water (mainly sand). The water then flows to the primary sedimentation tank (PST) where settleable solids are removed and the overflow of the PST, goes for biological nutrient removal (BNR). The BNR process is done by using bio-filters/trickling filters and the activated sludge process. The main parameters removed in the BNR process include phosphates, nitrate-nitrogen and ammonia nitrogen. As phosphates are not efficiently removed using the BNR process, chemical precipitation is effected

using ferric chloride. After BNR, the water flows to the secondary settling tanks (SST) and the overflow of the SST is disinfected using Ultraviolet (UV) or chlorine. The disinfected effluent is discharged into the Apies River[48].

Chemicals used to treat the water removes microbial agents, suspended particulate matter and some hazardous chemicals, but does not adequately remove pesticides and other endocrine disrupting chemicals. In fact according to the chief biochemist at Daspoort Wastewater Treatment Works, there is no process that removes endocrine disrupting chemicals from the wastewater; chemicals that would be removed are only those that are biodegradable[49].

Presently in SA, there is no wastewater treatment process that would be able to remove these EDCs. There is a major new EU funded research project over the next 3-5 years, jointly being carried out at the Chemical Engineering Department at the University of Bath, with academic partners in the UK, Finland, Israel and Germany, investigating the treatment of waste waters from the pulp/paper, herbicide/pesticide and halogenated organic chemical manufacturing industries, using technologies such as nanofiltration, electrodialysis, membrane bioreactors, extremophile organisms, ozonation and liquid-liquid extraction. The aim is to develop a waste water treatment process that will treat any toxic industrial chemicals and render them safe and will also attempt to recover or regenerate chemicals including water for reuse in the process[50].

An experimental bioreactor plant has been newly installed at Wessex Water's Porlock sewage treatment works. It is apparently highly efficient and represents one of the most advanced installation of its kind in Europe[50].

All the water samples collected in the Pretoria West study were positive for oestrogenic activity. The E_2 values of the samples range from 8.16×10^{-13} to 9.48×10^{-9} with sample 1 having toxic levels. Sample 1, was taken from a dense industrial area, with numerous small sized industries that, generate oestrogenically active chemicals as part of their processes (see table 1), or use chemicals such as

detergents in their cleaning processes and perhaps also create new compounds in their processes that may be oestrogenic in composition (see Appendix 1).

All the water samples tested positive for oestrogenicity. This is significant as this water is treated at the Daspoort Wastewater Treatment Works, then enters the Apies River, thereafter into the Bon-Accord Dam and finally into the Pienaars River. This is a very wide area that is contaminated with oestrogens, and may have far reaching implications for aquatic, animal and human health.

Presently there is no legislation on the permissible levels of oestrogen in drinking or other water; therefore it is not possible to state whether the levels are safe or dangerous. High toxicity levels detected in sample 1 is cause for concern.

The Apies River borders the Hammanskraal area, where there are some informal communities and settlements. Officially no persons should drink or use the water for household purposes directly from the river, but this may be occurring, if the communal taps are a long distance away. Taps in homes and communal taps have been provided in this area recently. Water is, however taken directly from the river for irrigation of farms and informal vegetable gardens. This is done with the knowledge and permission of the relevant authorities. These vegetables and fruit grown in these areas and watered with contaminated water, would again indirectly pose a health problem.

Rooiwal Power station which is further north, but still within the Tshwane boundaries, uses water from the Apies River for cooling purposes. This water is piped from the river, purified on site and then used for cooling.

The oestrogenic activity present in all the samples is as a result of the highly polluted industrial environment. This could impact detrimentally on the general health of the surrounding community as well as that of the workers in the small-sized industry itself. The endocrine disrupting chemicals (EDCs) which are responsible for the oestrogenicity of this water, may act adversely on the

endocrine system of various species, and humans. This may over time result in behavioural, nutritional and reproductive abnormalities, depending on the level, period and length of exposure.

Organochlorine Pesticides were detected in sample 7 and 3. In sample 3 a pesticide, Terbutylazine (TBA) was detected and again was not quantified, as it does not form part of the list of Organochlorine pesticides tested for.

TBA is not used extensively in South Africa, it is herbicide that belongs to the chlorotriazine family, is used both pre- and post-emergence treatment of a variety of agricultural crops and in forestry. Degradation of TBA in natural water depends on the presence of sediments and biological activity. Concentrations found in water seldom exceed 0.2µg/litre[51].

There is no evidence that TBA is carcinogenic or mutagenic. Guideline value for drinking water is 7µg/litre. This is calculated by allocating 10% of the TDI to drinking water[51].

Since TBA was not quantified, it is not possible to say whether the amount detected is significant or not. However the presence of a pesticide that is not extensively used in SA, in an area where there are predominantly small-sized industries, is of concern.

Sample 7 had 0.9 µg/L of Lindane, and unknown quantities of Chlorpyrifos and Atrazine. Sample 7 was taken at the entrance of the Apies River, after passing through the Daspoort Wastewater Treatment Works. The water at this point has been cleaned and can be considered safe for drinking and other uses as communicated by the city engineers that assisted in the collection of these water samples.

According to the European Union (EU) directive for drinking and ground water, pesticide levels must not exceed 0.1µg/L for individual compounds and some of

their degradation products; and the sum total of all pesticides should not exceed 0.5µg/L. The proposed guideline value for lindane in drinking water is 0.3µg/L as recommended by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) which conducted a recent review of lindane[52].

Lindane is used as an insecticide on fruit and vegetable crops (including greenhouse vegetables and tobacco) for seed treatment and in forestry. It is also used as a therapeutic pesticide (e.g. in the treatment of scabies and headlice) in humans and animals[52].

Lindane enters water from direct application for the control of mosquitos, from use in agriculture and forestry, from precipitation and to a lesser extent from contamination of wastewater from manufacturing plants[52].

Actions to ban or severely restrict lindane have been taken by 11 countries, while seven countries have banned the use of lindane. Among these countries Australia, Austria, Cyprus, Finland, Indonesia, the Netherlands, New Zealand and Saint Lucia cited persistency in the environment, bioaccumulation in the food chain and toxicity to humans, aquatic and terrestrial species as their reasons for banning or restricting the use of lindane. In South Africa, lindane has not been banned and is commonly found in certain pesticides, eg Effekto Woodborer, used to treat wood, and in over the counter medication to treat scabies and headlice[53].

In a United Nations Environment Programme, that was amended in 1996, all evidence on the health effects were reviewed by the World Health Organisation (WHO), International Agency for Research on Cancer (IARC), European Union (EU) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and it was found that long term use of 0.01 mg/kg bw of lindane, resulted in liver enlargement and slowly reversible kidney damage. A two year mouse oncogenicity study demonstrated increased incidences of liver tumours (male and female) when dosed at 20mg/kg/day throughout their lifespan. Overall lindane appears not to have mutagenic potential [52,53].

As there are many small industries, that manufacture products and a few households in the area, it is possible that lindane could have entered the underground water, from both domestic and industrial use.

Chlorpyrifos is a broad spectrum organophosphorus insecticide, used for the control of mosquitoes, flies, various crop pests in soil and on foliage, household pests and aquatic larvae. It is used as a soil treatment (pre-plant and at planting), a seed treatment, and as a foliar spray. Because chlorpyrifos is used as a mosquito larvicide in water bodies, it is desirable to establish a guideline value for drinking water. Assuming a 10% allocation of the acceptable daily intake (ADI) to drinking water, the proposed guideline value for chlorpyrifos is therefore 30µg/L[54].

In South Africa, chlorpyrifos is readily available and is found in over the counter pesticides, such as Effekto Ant, Effekto Chlorpyrifos and Kombat Chlorpyrifos.

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) has established an acceptable daily intake (ADI) of 0.01mg/kg body weight (bw) on the basis of the no observed adverse effect (NOAEL) of 1mg/kg bw per day for inhibition of brain acetylcholinesterase activity in studies in mice, rats and dogs, using a 100 fold safety factor, and on the basis of the NOAEL of 0.1mg/kg bw per day for inhibition of erythrocyte acetylcholinesterase activity in the study of human subjects exposed for 9 days, using a 10-fold safety factor.

Atrazine is a synthetic organic chemical classified as a triazine herbicide. It is used to suppress weed growth in edible crop production. The presence of atrazine residues in domestic water is of concern since there is evidence that atrazine may be carcinogenic in some animal species; this may indicate potential carcinogenicity in humans. The total tolerable dietary intake for atrazine is 0.5 * g/kg/bw/day[54].

In South Africa (SA) it is registered for use on maize, sorghum, and sugar cane. It may not be used on heavy clay soils. Registration for industrial use in SA was withdrawn in 1995. The US EPA has banned the use of Atrazine[55].

Chlorpyrifos and Atrazine were not quantified, but their presence in sample 7, which was the point where the water enters the Apies River has implications for both aquatic systems and surrounding communities.

Samples 1,3,5 and 7 had various levels of p-Nonylphenol, the presence of p-NP is not surprising as it is present in most plastic products and leaches into food and other consumables easily.

There has been no minimum guideline set in South Africa or by the European Union on permissible or allowable levels in drinking water for p-NP or the Nonylphenol group as a whole.

p-NP is a degradation product of nonylphenol polyethoxylates which are used in various industries for many years. p-NP is used as an additive or a surfactant in the manufacturing of plastics. The alkylphenol-polyethoxylates (APnEO) are a major group of surfactants and are normally present in raw sewage. Many of the products of biodegradation such as p-NP are both persistent and present in substantial quantities in effluent and in river water. This compound is capable of disrupting the endocrine system of animals, including humans and fish[56].

Annual usage of nonylphenol-polyethoxalates in Europe is around 75 000 tonnes. During sewage treatment, the APnEOs are biodegraded first into APnEOs with shorter ethoxylate chains and finally into alkylphenols themselves. The major pathways of this slow degradation process is summarised in Fig 1 below.

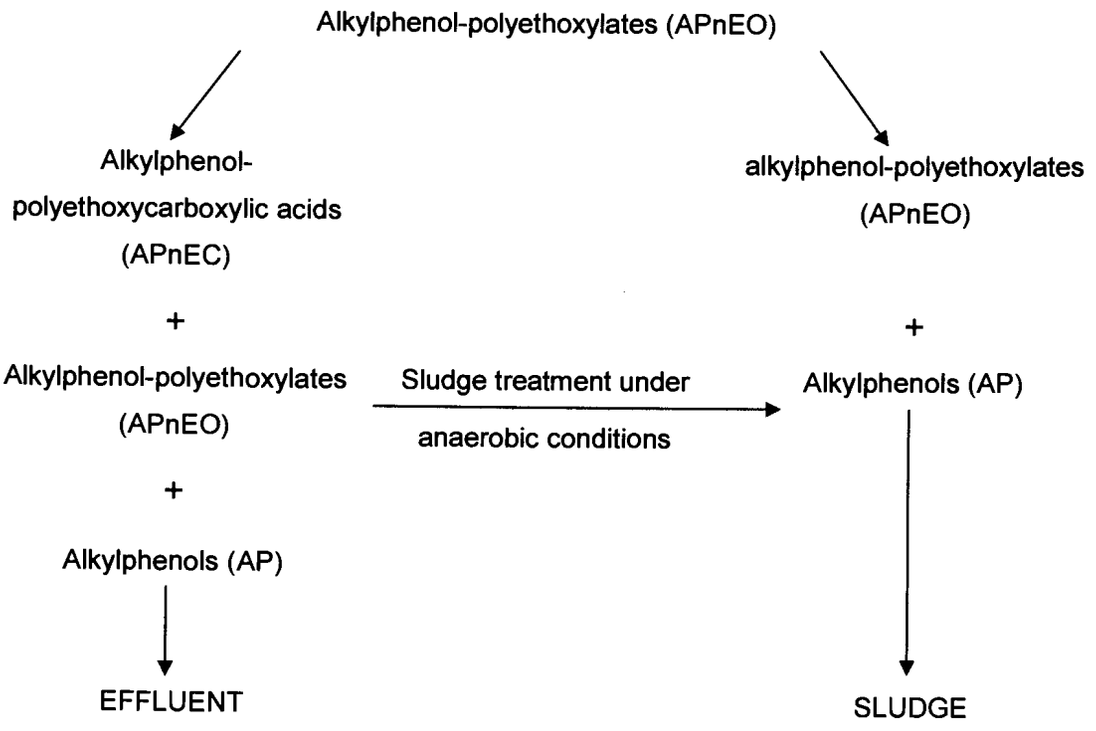


Fig 1. Biodegradation of surfactants and detergents during sewage treatment (based on the work of Giger et al. 1987).

During sewage treatment, 90% of the polyethoxylate compounds (APnEO) are eliminated. As the ethoxylate chain becomes shorter, the products become more resistant to biodegradation and more lipophilic. Because of their lipophilicity and persistence, alkylphenols bioaccumulate[56]. This may be the reason that p-NP was detected in sample 7.

For nonylphenol the low dose effects in F1 rats following dietary exposure (in utero through puberty) to 25ppm, include a decrease in volume of sexually dimorphic nuclei of the preoptic area (SDN-POA) of the hypothalamus in males, an increase in relative thymus weight, an increase in proliferation of the splenic T-lymphocytes stimulated with anti-CD3, and a prolonged estrus in females[57].

The phthalates tested for were DEHP, DBP and BBP. However no BBP was detected with the method used by the CSIR laboratories. Samples 1 to 7 had

various levels of DEHP, while DBP was detected in samples 1,3,4 and 6. Sample 6 had the highest levels of both DEHP and DBP. This was again not unexpected as this point was where all the water from the whole Pretoria West area converges. This point was according to the City Engineers and logic the most contaminated point.

There are no minimal levels set for phthalates in drinking or other water and thus, it is difficult to decide if the levels found in the various samples taken in the Pretoria West area are significant or not.

DBP is mainly used as a coalescing aid in latex adhesives. DBP is also used as a plasticizer in cellulose plastics and as a solvent for dyes. Release of DBP to the environment can occur during its production and also during the incorporation of the phthalate into plastics, adhesives, or dyes. DBP is not bound to the final product and as such can be released during the disposal or use of the product. This makes phthalates one of the most abundant man-made environmental pollutants[58].

Phthalates that are released to the environment can be deposited on or taken up by crops intended for consumption by humans or livestock and can thus enter the food chain. The main route of exposure is usually through food.

Levels of DBP in drinking water were estimated to be minimal. DBP exposure to adults through drinking water was estimated at 0.02 ug/kg bw/day by International Programme on Chemical Safety (IPCS) and Health Canada based upon a survey of drinking water supplies in Ontario, Canada and 0.005 ug/kg bw/day in adults as estimated by the Agency for Toxic Substances and Disease Registry (ATSDR) based on a survey of drinking water in 10 unspecified cities prior to 1986. Health Canada also estimated DBP exposures through drinking water intake in children and those values ranged from 0.022 ug/kg bw/day in children aged 12-19 years to 0.11 ug/kg bw/day in infants aged 0 – 6 months[58].

There are no data on the developmental or reproductive toxicity of DBP in humans. There are data from rat and mice studies that show oral exposure to DBP causes developmental toxicity. The developing male reproductive system is most sensitive to the formation of structural and functional abnormalities, with effects seen in rats whose mothers were exposed to 100mg/kgbw/day during pregnancy. The NOAEL for male reproductive system developmental effects in rats is 50mg/kg bw/day during pregnancy. Breeding studies provide a good indication of the potential for adverse functional reproductive effects from DBP exposure, such as Leydig cell hyperplasia and a low incidence of Leydig cell adenoma[58].

There are indications that oral exposure of females during the adult phase of life impairs functional reproductive performance in rats at doses of 250mg/kgbw/day and higher. Impairment in female fertility due to exposure during gestation and nursing has also been reported.

Data indicate that the monoester of DBP, Mono-n-butyl Phthalate (MBuP), is the principal toxicant. Studies suggest that an antiandrogenic mechanism appears to be responsible for the most sensitive endpoints observed in developing male rats (eg. Anogenital distance, nipple retention, preputial separation)[58].

DEHP is a general plasticizer in many PVC consumer products. Through these, DEHP has a ubiquitous presence in our environment. In the US it is no longer used in baby bottle nipples, teethers, or infant's toys. Exposure of the general population to DEHP occurs from food, water, and air, via inhalation and ingestion. The largest general population exposure to DEHP is dietary, followed by indoor air. In general, fatty foods including dairy, fish, meat and oils contain DEHP. The range of exposure in the general population from all sources, excluding non-dietary ingestion, medical and occupational, is estimated to be 3-30ug/kgbw/day[59].

There are very few human data from which to characterize the reproductive and developmental toxicity of DEHP. Therefore, evaluation of human reproductive risk must be extrapolated from studies in experimental animals where species

differences in metabolism and dynamics of PPAR-alpha are important considerations[59].

There are sufficient data in rodents to conclude confidently that oral exposure to DEHP can cause reproductive and developmental toxicity in rats and mice. These effects include malformations (tail malformations, axial and appendicular skeletal abnormalities, cardiovascular malformations, and neural tube closure defects), developmental delays, and intrauterine death. The lowest observed adverse effect level (LOAEL) based on malformations in rodents was 40mg/kgbw/day and a NOAEL of 3.7 – 14mg/kgbw/day was identified for testicular development / effects in rodents[59].

CHAPTER 5

5.1 CONCLUSION

The limited scope of the research does not allow for conclusive findings. However, there is strong evidence that small-size industries are contributing to pollution of the water in the Pretoria West area and subsequently polluting waters of surrounding areas and communities.

The water tested positive for oestrogens and specific endocrine disrupting chemicals. Due to the fact that there is at present no acceptable guideline values set for the majority of these EDCs tested; it is extremely difficult to state whether the levels found pose a potential health risk to humans, animals or aquatic life.

It was also noted that these EDCs are not removed by any of the processes of the water treatment works at Daspoort. In fact, in SA there is no water treatment plant that removes these EDCs, the only chemicals that will be removed are those that are biodegradable[49].

Presently drinking and other water is not tested for these EDCs, except organochlorine pesticides. Thus legislation for acceptable levels of these specific chemicals is not available.

Lindane an organochlorine pesticide was detected at a significant level in the water that had already passed through the Daspoort water treatment works. The level is above the acceptable daily intake limit for water and thus has the potential to create a health hazard both to the workers in the industry itself as well as to the surrounding communities. However the fact that lindane was not detected in any of the other water samples, more specifically in the sample where all the water from the greater Pretoria West area converges, needs to be investigated more carefully. This implies that lindane may have been introduced in the processes used by the

Daspoort Wastewater Treatment Works, which is highly unlikely. Other possibilities are that lindane may have not been detected by the detection methods used by the laboratories, although lindane has a very characteristic peak on the gas chromatograph and would be difficult to overlook. Further testing in this area is needed.

Further research, more specifically retrospective studies, where specific reproductive health outcomes in humans have been found are needed.

In view of the above, the work done by Toppari and team show that internationally and locally male reproductive health has received remarkably little attention considering that subfertility affects 5% or more of the men and that prostatic hypertrophy or cancer is a major problem for older men. It is now evident that several aspects of male reproductive health have changed dramatically for the worse over the past 30 to 50 years. The most fundamental change has been the striking decline in sperm counts in the ejaculate of normal men, the incidence of testicular cancer has increased progressively in many countries to become now the most common cancer in young men. Other disorders of the male reproductive tract may also be increasing in incidence, with several European countries reporting a progressive rise in hypospadias (a malformation of the external genitalia) and an apparently emerging trend toward an increasing incidence of testicular maldescent[1].

The book "Our Stolen Future" points out that many of the chemicals of greatest concern have already been banned in the U.S. but are being used in other countries. Thus we in South Africa should take greater responsibility to create and enforce legislation and better safety standards

It is difficult, if not impossible, for individuals to completely avoid hormone-disrupting chemicals because so many decisions about the use of chemicals are outside of our personal control. Action by industry and government is urgently needed to disclose product formulations, to reformulate products in order to

eliminate toxic substances; to accelerate the testing of thousands of chemicals that are currently on the market; and to pass tough legislation for controlling toxic chemicals.

Furthermore as suggested by Professor Pershagen of Stockholm, while epidemiological studies are crucial for assessing effects directly in humans and estimating population attributable risks their resolution power is limited, mainly because of difficulties in estimating exposure precisely and in controlling bias. Toxicological studies are necessary for elucidating causal mechanisms, which may be important for determining dose-response relations and extrapolation to low doses in risk assessment[60].

The solution to this huge, complex problem? Theo Colborn and Pete Myers suggested some beginning steps in their recent book, Our Stolen Future:
"Science alone does not always have the answer....The time has come to pause and finally ask the ethical questions that have been overlooked in the headlong rush of the 20th century. Is it right to change the Earth's atmosphere? Is it right to alter the chemical environment in the womb for every unborn child?"[61].

While the etiologies underlying these apparent changes are currently unclear, both clinical and laboratory research suggests that all of the described changes in male reproductive health appear interrelated and may have a common origin in foetal life or childhood. This means that the increase in some of the disorders seen today originated 20 to 40 years ago, and the prevalence of such defects in male babies born today will not become manifest for another 20 to 40 years or more[1].

A report from the National Research Council Meeting committee in 1999 agreed that although there is evidence of harmful health and ecological effects associated with exposure to high doses of endocrine disrupters, little is known about the harm posed by exposure to the substances at low concentrations, such as those that typically exist in the environment[8].

"Determining the risk to humans from contact with these chemicals in the environment is difficult because ordinary exposure to these agents has not been routinely monitored, and determining what these exposures actually are is therefore of primary importance", said committee chair Ernest Knobil, the Ashbel Smith Professor and H. Wayne Hightower Professor in the Medical Sciences, Medical School, University of Texas, Housten[62].

Another area of concern is that effects caused by exposure to endocrine disruptors during critical periods in development may not be predicted by studies conducted at later times in life (after weaning) and may not be detected by in vitro screens[62].

5.2 RECOMMENDATIONS

Governments in several countries have started to develop policies towards hormone disrupting chemicals. Most of these policies involve research and screening of chemicals, rather than regulatory action on individual chemicals. The chemical industry is unwilling to accept that endocrine disruption is more than a 'hypothesis'.

There needs to be more specific regulations on endocrine disrupting chemicals, more specifically regulation should be set for water, where permissible levels of specific chemicals in water should be set out clearly. This has been done for organochlorine pesticides as a whole, but many individual pesticides and other chemicals have no specific limits.

Pollution of the water by industry, especially small-sized industry which is under or not regulated at all, poses a serious threat to health, both to the workers in the industry itself, working with the various detergents, cleaning materials and chemicals and for the surrounding communities

. The manner in which effluent from industry is discharged into the water, has to be looked at carefully and perhaps an alternate system, or structure be put into place

that will reduce the hazardous chemicals, that are being discharged into the water. The Department of Labour needs to monitor this strictly.
Unnecessary exposures should be reduced. These hazardous exposures should be reduced for workers in small-sized industry as well as the surrounding communities, by encouraging small industry and government to jointly find alternate means of disposing of waste and attempting to reduce the exposures to these various chemicals as far as possible.

Enhanced technical processes for the treatment of domestic, agricultural and industrial waste have to be researched and found. A major project comprising the use of membrane bioreactors to treat industrial and other waste is underway at the University of Bath and this could be a breakthrough for us in the near future.

Other technologies such as increasing the sludge age of the effluent that passes through the water treatment works have to be investigated and perhaps put in place. These may not remove all the EDCs but may help to remove the slowly biodegradable organic matter, which the present water treatment systems do not do.

This requires commitment on the part of government and substantial funding, which is not always available.

As individuals and as a society we should also try and eliminate unnecessary exposures to known and likely endocrine disrupting chemicals, particularly during vulnerable periods such as during pregnancy and childhood[63].

There are many actions that consumers can take to reduce their exposure to hormone disrupting chemicals, both through buying different products and by pressurising companies and governments to phase them out. It is virtually impossible, however to avoid all exposure, as these chemicals are extremely widespread.

Curtail the introduction of thousands of new synthetic chemicals each year, reduce the use of pesticides as much as possible and pesticides should be used only in genuine emergencies.

Shift the burden of proof onto manufacturers. The current system assumes that chemicals are innocent until proven guilty. This is wrong. The burden of proof should work the opposite way, because the current approach, has time and again made people sick and damaged ecosystems.

Governments should set current legal limits of these various chemicals at low enough levels to protect human health and eliminate exposure to wildlife where practicable. The World Wildlife Foundation (WWF) considers that the public should be given access to all research findings, and regulators should aim to honestly inform the public about the concerns and uncertainties with regard to the effects of specific chemical exposures. Furthermore, the public should be given the right to know about the constituents of products, in order to enable them to make informed choices.

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Appendix 1 – Detergents used by industries in study area

Industry	Detergent	Area to be cleaned
Meaty Pies	Oven cleaner	Ovens
Meaty Pies	Heavy duty pine gel	Floor
Meaty Pies	No name dishwasher	Dishes
Meaty Pies	Tiolet bowel cleaner	Tiolets
Meaty Pies	Jeyes Fluid	Drains
Dups Scrapyard	Sulight liquid	Dishes
Dups Scrapyard	Harpic/Handy Andy	Toilet
Dups Scrapyard	Greasecutter	Engines, web machine
Dups Scrapyard	Omo, Multipurpose cleaner	Car body
Dups Scrapyard	Handcleaner (wholesalers)	Wash Hands
Tip Top Café	Persal (Trade Centre)	Floors, ovens, dishes, etc
Claudinos Pizza	Pan release (Chipkin, Jhb)	Pans
Claudinos Pizza	Green Dishwashing liquid (Cater sales)	Dishes
Claudinos Pizza	Javel	Sanitizer
Early bird service	Servicesol	Switchcleaner
Early bird service	surgical spirits	
Early bird service	Commercial detergents	Floors
D & J Service Station	Handy Andy	Floors
D & J Service Station	No name soap powder	Driveway
D & J Service Station	Econo liquid wax and wash	Cars
D & J Service Station	Commercial window cleaner	Windows
Magic Photos	Chemical cleaners (Photo Ease Chem Cor product)	Grease on photographic and printing machine
Magic Photos	Wonderclean (Checkers)	Floors
Magic Photos	Mr Muscle	Counters and windows

T & M Motor Repairs and services .	Hand Cleaner (No name brand - buys it from a person who sells it door to door)	Cleaning of hands
T & M Motor Repairs and services	Engine cleaner	Engines and floors
Mr Cash and Mr Valet	Connex	Engines
Mr Cash and Mr Valet	Shampoo	Car body
Mr Cash and Mr Valet	Heavy duty	Car seats
Church Street Motors	Engine cleaner (buys from a person who comes door to door)	Engines
Church Street Motors	Car wash gel	Cars
Church Street Motors	Hand cleaner	Hand washing
Precision Autobody	Car wash and wax (Quality clean, Buitekant Street)	Washing and waxing
Precision Autobody	Washing powder	Car body
Precision Autobody	Floor Cleaner	Floors
Supercare Autobody	Household liquid soap	Car body
Supercare Autobody	Handy/ Jik	Floors
Supercare Autobody	Diesel	Engine grease removing
Sao Tiago Café	Family Favourite (Drug Centre)	Fryers, pans, oven
Sao Tiago Café	Washing Powder	Floors
Enzos Panel Beaters	Sunlight Liquid	Car body
Enzos Panel Beaters	Steam cleaning	Engines
Enzos Panel Beaters	Valet	Interior of cars
All Power	Momar products or sales	Grease from machinery
Lusio Autobody Repairs	Omo/Surf	Car body and grease
Lusio Autobody Repairs	Car sprays and cockpit sprays (Makro)	Interior, rims, tyres
Eljoney Executive Body Repairs	No name brand green liguid (bought from city centre)	All purpose
Precision Autospray	Car Shampoo(Marie Daniel	Interior and exterior

	Chemochem, Onderstepoort)	
Precision Autospray	Engine Cleaner - Action, Harveys	Grease removal
Body and Panel	Engine cleaner	Engines (Multi Maintenance Products)
Body and Panel	Washing powder	Car body
Body and Panel	Car Shampoo(Marie Daniel Chemochem, Onderstepoort)	Interior
Body and Panel	Hand cleaner	Hand washing
Dirks Panel Beaters	Household Washing powder	Car body
Dirks Panel Beaters	PH Polish	Polishing of cars
Jackaranda Panel Beaters	Surf	Car body
Jackaranda Panel Beaters	Valet	Bigger Jobs
Progress Panel Beaters	Safic Dishwasher	All purpose Wash
Gilos panel Beaters	Car wash and wax	Washing and waxing of cars (Marpro Zap)
Gilos panel Beaters	Engine cleaner	Grease removal
Gilos panel Beaters	Hand cleaner	Washing of hands
Gilos panel Beaters	Silicone spray	Dashboard
Italian Panel Beaters	Liquid Clean (Quality Clean)	Car body
Italian Panel Beaters	Engine cleaner	Engine
Toria Panel Beaters	Multipurpose cleaner (Rodita Manufacturers)	Car body
Toria Panel Beaters	Car wax	Waxing exterior
Toria Panel Beaters	Dishwashing cleaner	General cleaning
Toria Panel Beaters	Stripper (Harveys, other car accessory places)	Strong degreaser
Competition Motors	Win Penn Engine Cleaner	Engine grease removing
Competition Motors	Dishwashing liquid	Car body
Landmans Garage	Washing powder (Bought from home manufacturers - No	Car body

	name)	
Landmans Garage	Engine Detergent	Engine cleaner
Western Bikes	Engine Cleaner (Spare Shops)	Engine grease removing
Western Bikes	Paraffin	Stripping of grease
Valhala Service Station workshop	Will Penn Auto lubricants	Engine degreaser
Valhala Service Station workshop	Prepsol degreaser	Floors
Invector Autobody	Sunlight Liquid	Car body
Invector Autobody	Degreaser (Viva Spares)	Grease removal
Targa Panel Beaters	Dishwashing Liquid (Zircon Cleaning Products)	Car body
Targa Panel Beaters	Spot remover/cleaner	Interior of cars
Targa Panel Beaters	Engine Cleaner (One stop motors)	Grease removal from cars
Dairy World	HCl	Pasteurizing milk containers
Dairy World	Caustic Soda	Cleaning of machinery and pipes where milk runs thru
Dairy World	Hydrogen Peroxide	Surface of tables in the process plant
Dairy World	Chlorine and Jik	Plastic drums, lids - sanitizes
Dairy World	Iodine	Rinsing of pipes and washing of hands
Dairy World	Steam	Sterilization

Appendix 2 – Compounds tested during Biochemical Analysis

Organochlorine pesticides

1. Alpha – BHC
2. Beta – BHC
3. Gamma – BHC (Lindane)
4. Delta – BHC
5. Heptachlor
6. Aldrin
7. Heptachlor Epoxide
8. Endosulfan I
9. 4,4 – DDE
10. Dieldrin
11. Endrin
12. Endosulfan II
13. DDD
14. Endrin Aldehyde
15. 4,4 – DDT
16. Endosulfan Sulphate
17. Endrin Ketone
18. Metoxychlor

Polychlorinated Biphenyls

1. 1,1'-Biphenyl, 2,4'-dichloro- (PCB8)
2. 1,1'-Biphenyl, 2,4,4'-trichloro- (PCB28)
3. 1,1'-Biphenyl, 2,3,3'-trichloro- (PCB20)
4. 1,1'-Biphenyl, 2,2',5,5'-tetrachloro- (PCB52)
5. 1,1'-Biphenyl, 2,2',4,5,5'-pentachloro- (PCB101)
6. 1,1'-Biphenyl, 2,3,4,4',5-Pentachloro- (PCB118)
7. 1,1'-Biphenyl, 2,2',4,4',5,5'-hexachloro- (PCB153)
8. 1,1'-Biphenyl, 2,2',3,4,5,5'-hexachloro- (PCB138)
9. 1,1'-Biphenyl, 2,2',3,4,4',5,5'-heptachloro- (PCB180)

Nonyl Phenol

1. p-Nonyl Phenol

Appendix 3 – Baseline Questionnaire - Occupational Health In Small Industries - Pretoria West

General Information

1.1 Date: _____

1.2 Name of person completing this questionnaire:

_____

1.3 Position in the company:

_____

1.4 Name of industry / business / company

_____

1.5 Nature of Industry?

_____

1.6 Physical Address of industry / business / company?

_____

_____

1.7 Tel. Number: _____

1.8 Fax Number: _____

1.9 Cell Number: _____

1.10 E-mail: _____

2. Demographic Data of workforce

2.1 Please give the number of employees in your workplace (today)?

Males	
Females	
Total	

2.2 How many employees have left your employ during the last year?

	Numbers of employees
Retired - because of age	
Retired - medical reasons	
Discharged	
Retrenched	
Died	
Transferred	
Resigned	

2.3 How many new employees were employed to replace the ones who left?

_____

2.4 How many hours a week do your employees work?

Number of hours	**Numbers of employees**
< 40 hours	
40 – 49 hours	
> 50 hours	

3. Description of Industry

3.1. What are the most important outputs of your company (e.g. aluminium pipes, electric motors, assorted biscuits, panel beating, spray painting, exhaust repairs, etc.)

3.1.1 _____

3.1.2 _____

3.1.3 _____

3.1.4 _____

3.1.5 _____

3.1.6 _____

3.1.7 _____

4. Health Service information

4.1 Do you provide a health service on your premises (please tick)?

Yes	
No	

4.2 If no, then where do your employees get health care (please tick)?

4.2.1	Private general practitioner	
4.2.2	Private Health Centre, eg. Medicross	
4.2.3	Local government clinic	
4.2.4	Pretoria Academic Hospital	
4.2.5	Kalafong Hospital	
4.2.6	Pretoria West Hospital	
4.2.7	Traditional healer	
4.2.8	Other, specify	

4.3 If yes, then what type of health service is provided on the premises?

4.3.1	Nurse visits premises	
4.3.2	Doctor visits premises	
4.3.3	Employees attend clinic or hospital	
4.3.4	Employees attend doctors consulting rooms	
4.3.5	Other, please specify:	

5. General Information

5.1 Are there any union/s operating in your business?

Yes	
No	

5.2. If yes, please specify (in order of membership)

5.2.1 _____

5.2.2 _____

5.2.3 _____

Continuation Sheet - Occupational Health In Small Industries - Pretoria West

1.1 Date: _____

1.2 Name of person completing this questionnaire:

_____

1.3 Position in the company:

_____

1.4 Name of industry / business / company

_____

1.5 Physical Address of industry / business / company?

_____

_____

6. Use of detergents

6.1 List the various different types of detergents being used in your industry and its uses

Types of detergents	Uses

6.2 Where do you throw the water, after cleaning with detergents

_____

_____

_____

_____