

**THE QUALITATIVE AND QUANTITATIVE EVALUATION
OF ESTROGEN AND ESTROGEN - MIMICKING
SUBSTANCES IN THE SOUTH AFRICAN WATER
ENVIRONMENT**

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 **Nothing in life is ever given to you except by your loving parents,
but whatever you want to achieve in life, you have to work for.**

Anonymous 

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Abstract

The qualitative and quantitative evaluation of estrogen and estrogen-mimics in the South African water environment.

Recently some chemicals has been accused of mimicking the female hormone estrogen. The mode of action is termed *estrogen-mimicking*, because of the effects that is produced by these chemicals. Playing canary in a coal mine, wildlife was the first to indicate that something was wrong. Reproductive abnormalities such as abnormal sperm, reduced sperm production, cryptorchidism (undescended testes), abnormal hormone levels - particularly high estrogen ratios to that of testosterone, infertility, and genital abnormalities were all evidence that something in the environment was affecting the reproductive status of these animals. Also in humans instances like rising cases of infertility, abnormal sperm conditions and cases of breast cancer raised an increased concern about the potential disruptive effects of these substances on the human existence. After investigations were conducted, it was found that substances which enhance the quality of life like some detergents, agricultural products, additives to plastic, dyestuffs, paint components and pharmaceuticals to name but a few, all caused an abnormal reproductive status as seen in animals. The aims of this study were not to focus on the effect of these substances, but rather to identify estrogen and estrogen - mimics in the South African environment and to evaluate their presence in the water environment qualitatively and quantitatively with the most suitable analytical methods. Estrogen - mimics such as nonylphenol, o'p' and p'p' DDE, o'p' DDD, lindane, atrazine and the PCB's : Arochlor 1254 & Arochlor 1260 were found to contaminate the environment. The drinking water tested of the PWV area indicated that the water was free of these substances, and thus indicated a present safe situation.

Keywords : estrogen, estrogen - mimics, determination, South African Water environment, drinking water, industrial effluent.

Die kwalitatiewe and kwantitatiewe evaluering van estrogeen en estrogeen nabootsende stowwe in die Suid Afrikaanse water omgewing.

Onlangs is chemiese stowwe daarvan beskuldig dat hulle die vroulike hormoon estrogeen, naboots. Die meganisme van werking is *estrogeen nabootsend* vanweë die feit dat die stowwe estrogeenagtige effekte toon. Soos 'n kanarie in 'n steenkool myn, was die diereryk die eerstes wat aangedui het dat iets in die natuur nie pluis was nie. Reproductiewe abnormaliteite soos verlaagde sperm produksie, abnormale sperme, kriptogidisme (onuitgesakte testes), abnormale hormoon vlakke - veral verhoogde estrogeen - testosteroon verhoudings, genitale afwykings en abnormaliteite het alles as bewyse gedien vir dié verlaagde reproductiewe status. Ook die mens was nie uitgesluit nie, soos verhoogde insidente van infertiliteit, abnormale spermproduksie en 'n toename in borskanker groeiende aandag oor die potensiële invloed van hierdie stowwe op die menslike bestaan gehef het. Nadat 'n ondersoek ingestel was, was gevind dat van die stowwe wat die kwaliteit van die lewe verhoog het soos industriële en huishoudelike produkte geïmpliseer was. Dit sluit stowwe soos oplosmiddels, landbou produkte, stowwe wat gebruik word in die plastiese bedryf, kleurstowwe, verfbestanddele en farmaseutiese produkte, om maar 'n paar te noem, in. Die doel van die studie was nie gefokus op die effekte van die estrogeen en estrogeen nabooters nie, maar liever op hul voorkoms in die Suid Afrikaanse water omgewing en die evalueering van dié voorkoms in kwalitatiewe en kwantitatiewe terme deur middel van die mees geskikte analitiese metodes. Estrogeen nabooters soos nonielfenol, o'p' DDE, p'p' DDE, o'p' DDD, lindane, atrasien en die twee PCB's : Arochlor 1254 en Arochlor 1260 was gevind as van die stowwe wat die omgewing gekontamineer het. Drinkwater wat getoets was van die PWV gebied het die afwesigheid van die stowwe aangetoon wat dui dat die huidige situasie veilig is.

Kernwoorde : estrogeen, estrogeen nabooters, bepaling, Suid Afrikaanse water omgewing, drink water, industriële afval



Introduction

Disrupters of estrogens, false estrogens, gender benders, endocrine disrupters, environmental estrogens, ecocancers, malignant mimicry, estrogen pollutants, and hormone modulators. All of these are terms that describe estrogen and estrogen - mimics. During the discussions that follow the term *estrogen and estrogen - mimics* will be used.

Much concern has been raised about possible adverse consequences arising from release into the environment of substances with estrogenic properties. Possible effects on humans include reproductive disorders such as reduction in sperm count and quality, cryptorchidism (undescended testes), testicular cancer and male breast cancer. Environmental effects such as masculinisation of female fish, occurrence of female yolk protein, vitellogenin, in male fish and developmental abnormalities in reptiles have also been observed and there is evidence that these effects can be elicited by estrogenic chemicals released into the environment. The effect that these substances have on the reproductive systems of both human and animal life is well documented in the literature (Begley & Glick, 1994:44; Bergeron *et al.*, 1994:780-781; Best *et al.*, 1994:697-702; Bishop *et al.*, 1991:521-548; Bull, 1980:3-21; Carlsen *et al.*, 1992:609-612; Colborn, 1991:85-152; Cooke, 1973:85-152; Cooper, 1991:463-495; Davison & Sell, 1974:222-232; Deeming & Ferguson, 1988:19-39; Dibb, 1995:27-31; Dutta *et al.*, 1994:215-220; Elliot *et al.*, 1989:91-114; Espir *et al.*, 1970:423-425; Faber & Hickey, 1975:27-36; Faber & Hughes, 1993:35-39; Facemire *et al.*, 1995:79-86; Falck *et al.*, 1992:143-46; Fox, 1992:147-158; Fry & Toone, 1981:43-44; Giesy *et al.*, 1994a:128-135; Ginsburg *et al.*, 1994:230; Giwercman & Skakkebaek, 1992:3-9; Giwercman *et al.*, 1993:65-71) ranging from decreased sperm production, abnormal sperm, infertility, increased cases of

breast cancer in males and females, abnormally high levels of estrogen, genital abnormalities, lower reproductive status and cryptorchidism (undescended testes).

Chemicals implicated into causing estrogenic effects or generally endocrine disrupting effects include many substances commonly used in the industrial and household products which enhance the quality of life. Such substances, as stated above, include some detergents, agricultural pesticides, additives to plastic, dyestuffs, paint components and pharmaceuticals.

For a detailed list of the chemical names and classification of the various estrogen and estrogen-mimics refer to appendix 1, and for a detailed table of the various usage and applications of the estrogen and estrogen-mimics refer to appendix 2. Appendix 3 gives an indication of the structures of some of the estrogen and estrogen - mimics.

Whichever way you look at it, estrogen and estrogen - mimics are all around us. They are used as **herbicides** (Hileman 1994:19-23; Thomas *et al.*, 1993:147-151;), **pesticides / insecticides** (Hansen & Jansen, 1994: 525-527; Raloff, 1984:56-58) **organochlorinated pesticides** (ANON, 1985:127; Melius, 1995:77-79; Raloff, 1984:24-27; Scafferner *et al.*, 1983:132), they are used as **dielectrical fluids in transformers** (Hileman, 1994:19-23; Raloff, 1984:56-58; Sager & Girard, 1994:52-74;) in **kiss proof lipsticks** (Hileman, 1994:19-23; Lee Davis & Bradlow, 1995:144-149), in the Industry they are used as **plasticizers** in the production of vinyl floors (ANON, 1995a:47), in the **manufacture of plastics, antioxidants, during paper manufacturing** (Raloff, 1995:44-46), and as **an industrial solvent** (Raloff, 1993:10-15). They are used as **disinfectants**, and are found in **fungicide and germicide products** and are formed during the **combustion of foods and cigarettes** (Weiss, 1994:169-175). In the food industry they are used to **carbonate cooldrinks** (White *et al.*, 1994:175-182), and the pharmaceutical uses include the **treatment of stomach ulcers** (Lee Davis & Bradlow,

1995:144-147), **asthma remedies** (ANON. 1994a:442; Re *et al.*, 1993:438-442) as **fungicides** (Perez *et al.*, 1993:233-243), **neoplastic agents** (ANON., 1993c:21-24), **anabolic agents** (Leroy *et al.*, 1988:453-458; Mueller & Kim, 1978:1429-1435), **vitamin supplements** (Nielsen *et al.*, 1992:237-246), to **relieve menopausal symptoms and as oral contraceptives** (Aherne & Briggs, 1989:735-736; De Vito *et al.*, 1991:321-338; Dibb, 1995:27-31; Purdom *et al.*, 1994:275-285; Raloff, 1984:24-27). They are also **present in some plant species** (Field *et al.*, 1990:44-54; Ginsburg, 1994:230; Vines, 1993:5) and has been traced in tinned peas, artichokes, corn and mushrooms, the source being bisphenol A, a **chemical lacquer on the inside of food tins** (ANON., 1997:127).

This study was mainly done because there is very little information about the occurrence of estrogen and estrogen-mimics in the South African water environment.

The *aims and objectives* of this study is to assess the qualitative and quantitative extent of contamination of the South African water environment.

In brief the aims and objectives were :

1. To conduct a comprehensive literature survey to identify specific chemicals which have been claimed to mimic estrogens.
2. Based on the information obtained in the literature survey, identify which of those compounds are most likely to occur in the South African situation.
3. Evaluate which of those substances are the most economically important and analytically feasible.
4. Determine the present state and location of contaminants. This information will enable the authorities to formulate new strategies to monitor water sources and investigate new treatment methods if necessary.

The project was initiated by Rand Water and is sponsored by the Water Research Commission (WRC).

The objectives were achieved by taking the following steps :

1. To conduct a literature survey, making use of databases such as Toxline, Chemdata, Waterlit and Medline. The literature survey was further complemented by many overseas articles that were obtained via the Potchefstroom University's interlibrary loan system, as well as other sources that were found in articles, books and magazines that were gathered throughout the two years of study.
2. The present level of contamination was assessed by the literature survey as well as information gathered from Water authorities, Government departments such as the Department of Water and Forestry, laboratories such as Cydna Laboratories, Okkie Fourie Toxicologists and Infotox.

Samples were collected and analysed to determine the levels of contamination by the specific chemicals.

Chapter**1****Estrogens in the environment**

Thousands of chemicals are introduced into our environment on a daily basis with little knowledge of their effects on human reproduction and development. The overall human exposure to these chemicals are also not known. With millions of women taking oral contraceptives and other hormonal treatments, environmental contamination is a distinct possibility.

Humans now live in an environment that can be viewed as a virtual "sea of estrogens". The environmental load of estrogens can easily be illustrated by the following : In 1970 alone, over 27000 kg of Diethylstilboestrol (DES) was used for poultry, cattle, and sheep as a growth-promoting agent (Rall & McLachlan, 1980:199). The known adverse effects of prenatal exposure to this substance are well documented (ANON., 1993b:11; ANON., 1995e:933-935, Bilbo *et al.*, 1977:1-8, Faber & Hughes, 1993:35-39, Holden, 1993:1238-1239, Sharpe & Skakkebaek 1993:1932-1395, Stillman, 1982:905-921, Stone, 1994:499-500). o'p' DDT, an isomer of DDT, which accounts for 15-20% of the commercial mixture of DDT, was estimated to have been used up to 1980 , in as much as 200 million pounds per annum in the USA. Another series of compounds that are structurally related to DDT, is a group called poly chlorinated biphenyls or PCB's. These compounds have been widely used in additives of paints, plastics, rubber, adhesives, printing inks, and insecticides. PCB's have been reported to reach the fetus after maternal exposure in a number of species, including humans (Rall & McLachlan, 1980:200). Polycyclic aromatic hydrocarbons, or PAH's are widespread environmental contaminants resulting from the combustion of fuels. It should be noted that some of the chemicals that have been shown to have estrogen activity are also found as naturally occurring substances in the environment.

Examples of these are zearalenone, which is a product of the fungus, *Fusarium*, which commonly infests corn. Another group is the phytoestrogens. Examples of some of these substances are genistein and coumestrol. Both of these substances have been shown to have interactions with the cytoplasmic receptor of estradiol (Rall & McLachlan, 1980:200). An example of the exposure to these estrogen-mimics are the outbreak of infertility in sheep that were grazing on subterranean clover in Australia. After an investigation was made, genistein was isolated from the clover eaten by the sheep (Rall & McLachlan, 1980:200). Tetrahydrocannabinol, (THC), one of the active components of marijuana, was also found to be estrogenic, as there were reports of incidences of gynecomastia (Rall & McLachlan, 1980:200).

It is estimated that 1.5 million tons of PCB's have been produced globally since the 1930's. Calculations suggest that about 20-30% of all produced PCB's in one way or another have found their way into the environment, accumulated in dumpsites or sediments of lakes. Although global PCB production has stopped, more than 70% is still in use and could reach the environment in the future. It is shown that approximately 1% of the total PCB quantity has reached the open ocean (Reijnders & Brasseur, 1992:168).

Thus, it is clear that we are exposed to substances with varying degrees of estrogenic activity on a daily basis.

1.1 Impact of environmental estrogens on the animal kingdom.

Playing canary in a coal mine, wildlife was the first to send out signals that something was seriously wrong in the animal kingdom. The following discussion will illustrate the impact that the estrogen and estrogen - mimics have on the reproductive system of the wildlife.

During the early 1990's, Professor Louis Guillette Jr., of the University of Florida, USA, had to determine how many alligators could be hunted down from Florida's Lake Apopka, the South's largest body of freshwater, without making the population crash. During the investigation, he noted that these alligators had significant abnormalities of their reproductive systems. In general reproductive disorders reported involved matters like reduced fertility, reduced hatchability, reduced viability of offspring, modified hormone activity, or altered sexual behaviour (Guillette *et al.*, 1984:680, Lutz, 1996:13). Female alligators of six months old, had plasma estradiol levels nearly twice that of normal females. Abnormal ovarian morphology with large polyovular follicles, and polynuclear oocytes was also exhibited by the females. The males all had abnormal penises, being one quarter the normal size, poorly organised testicles and the testosterone levels of the juvenile male alligators compared well to that of females of the Lake Woodruff surrounding, indicating a serious case of sterility (Begley & Glick, 1994:44, Guillette *et al.*, 1984:680). After an investigation followed, it became evident that in 1980, thousands of litres of Kelthane, a DDT - containing pesticide - and dicofol, was spilt into the Lake (Begley & Glick, 1994:44, Guillette *et al.*, 1984:680, Lee Davis & Bradlow, 1995:149), resulting in the reproductive abnormalities that was seen in the surrounding wildlife. Further contamination was also derived from extensive agricultural activities around the lake, as well as a sewage treatment facility associated with the city of Winter Garden, Florida, USA.

Also at Lake Apopka, Red-bellied sliders, a common turtle species, that live among the alligators, showed signs of feminisation, and was shown to be reproductively incompetent (Gills, 1994:297).

Vitellogenesis, the production of vitellogenin, a precursor of yolk, is usually found in all egg laying animals after a stimulus is sent by estrogen to the liver. Thus, the presence of vitellogenin in plasma is indicative of estrogen stimulation to the liver. The rainbow trout and carp found in the waterways of England and Wales were hermaphrodite, and all displayed plenty of

vitellogenin - even the male fish (Raloff, 1984:26). This led to a study where fish were caged in the effluent of sewage - treatment works, and one to three weeks later, the vitellogenin was measured. The results obtained from this study indicated a pronounced increase (500 to 100000-fold, depending on the site) in the plasma vitellogenin of the fish. It was suggested that the most likely estrogenic substance(s) were ethinylestradiol, originating from the pharmaceutical use, or alkylphenolic ethoxylates (APE), originating from biodegradation of surfactants and detergents during sewage treatment (Purdom *et al.*, 1994:275). The concentrations of the ethinylestradiol was found to be as low as 0.1 ng/l.

The Pallid sturgeon, an endangered fish native to the Missouri and Mississippi Rivers, have shown no record of reproduction. All the pallid sturgeons that are seen today, are 30 to 40 years old. High concentrations of PCB's and DDT have been found in the pallid sturgeon. Furthermore, the gonads of the sturgeons were found to be, in the 15 years of investigation, not distinctly male or female (Raloff, 1984:26).

The death of embryos, deformities and abnormal nesting behaviour in fish-eating birds living in the Great Lakes regions contaminated by chlorinated organic compounds were observed. For example, eagles and other birds have been born with crossed beaks; female herring gulls have been found to share nests with other females, because the males were influenced by estrogen mimicking substances, and hence had no sexual inclination (Lee Davis & Bradlow, 1995:149).

Environmental estrogens are also suspected to play a role in the reproductive problems of the Florida panther. During the period of 1985 to 1990, 67% of the males were born with one or more undescended testes, a condition known as cryptorchidism. 10 years previously, only 14 % of the males were cryptorchid. At least one non-cryptorchid male was sterile, while normal males presented with abnormal or deformed sperm. After an investigation of the

blood levels of the panthers, it became evident that one male had twice as much estrogen as testosterone, and one female presented with more testosterone than estrogen. In the surrounding Florida area, the soil and water contained high concentrations of heavy metals, and persistent chlorinated organic substances (Lee Davis & Bradlow, 1995:149).

DDT wreaked havoc among birds which are normally resistant to eggshell thinning, such as seagulls. Though heavily contaminated gull embryos managed to hatch, reproduction in gull colonies exposed to large amounts of DDT began to decline in the late 1960's (Raloff, 1984:24). It was also noted that the young among these communities bore grossly feminized reproductive tracts. Female gulls, which should have mature reproductive organs only on their left side, also carried oviducts on their right side. Many males also bore feminine characteristics such as oviducts; the males had tissues that were both ovarian and testicular - so the birds became an intersex (Raloff, 1984:25).

Laboratory studies on fish at the University of Guelph in Ontario, has shown that White suckers exposed to papermill effluent - often rich in dioxins and related compounds - took longer to mature, developed smaller gonads, experienced reduced fertility and had lower than normal concentrations of steroid hormones in their blood (Raloff, 1984:27).

Other researchers are linking reproductive problems in Salmon to relatively high concentrations of hormone-like contaminants (Raloff, 1984:27).

Raloff (1984:24-27) reported a study that was done on the effect that estrogenic pesticides and PCB's have on the terns, a relative of the gull. The study concluded that the male terns had varying degrees of feminized sex organs (Raloff, 1984:25).

Research in Texas has shown that turtles hatched from eggs to which shells had been applied a low dose of estrogen-mimicking substance, ovaries were forming in turtles that were otherwise male (Dibb, 1995:28).

Western gulls on the coast of California and herring gulls in the Great Lakes show abnormal breeding behaviour, apparently as a result of organochlorine exposure. During the 1970's, when the pollution of DDT was at it highest, bird populations in both areas showed abnormally high numbers of eggs in every nest, and pairing between female birds. The large clutches were due to the joint rearing of broods by several birds. As the contamination level of DDT declined, the abnormal behaviour disappeared (ANON., 1993c:23).

Sherry E. Reir, of the South Florida College of Medicine, USA, reported that rhesus monkeys that were exposed to large doses of dioxin had higher rates of endometriosis than the unexposed monkeys (Weiss, 1994:12).

During the late 1960's a director of the National Institute of Health (NIH) laboratory at Helena, Montana, USA complained about his previous fertile mouse breeding that had become virtually non-productive during the preceding two months. He also noted that the male mice had developed inguinal hernias that they then dragged behind them as a scrotally placed mass. These features suggested that the problem might be a dietary source of estrogen. After an intensive investigation they found out that the pellets that the mice was fed on, contained a uterotrophic substance in sufficient concentrations to provide a fully effective dose in a few day's ration.

Another interesting occurrence took place with untreated control rats that started to show uterine enlargement. Careful review of this brought to light that the rats were dusted with insecticide powder in order to keep the infestation of ectoparasites to a mimimun. The composition of the powder was determined, and the estrogenic substance, Methoxychlor, was found. The

animals ingested this by grooming themselves and their cage partners (Hertz, 1980:347-352).

Other incidences were the deformation of shells in oysters harvested from kepone-contaminated waters (Lee Davis & Bradlow, 1995:149). Twice the rate of testicular cancer and reproductive defects in military dogs that served in Vietnam, compared with dogs that served elsewhere during the same period (Lee Davis & Bradlow, 1995:149). It was shown that DDT can turn on vitellogenin production in the male red-eared turtle, one of America's most common turtles (Raloff, 1984:26). The pesticide, chlordane (commercial name, Kepone, or CD), was used extensively as an agricultural pesticide, and causes sperm malformation, and blocks the reproductive function in many birds and mammals (Srivastava & Srivastava, 1994:186).

In a recent article of Warhurst (1995b:92), he commented on the problem of the estrogenic effects of the alkylphenolic compounds, and a phase out program that was initiated to rule out the group of the nonylphenol ethoxylates (NPE's) that is extensively used as industrial cleaning agents. However the other group : the octylphenol ethoxylates (OPE's), that has only a tenth of the sales of NPE's, are ten times more estrogenic than nonylphenolic compounds. However this phase out ignores other uses of alkylphenolic compounds and is thus clearly inadequate. Both of the above mentioned compounds have been shown to affect wildlife and could be affecting human health.

1.2 The impact of environmental estrogens on the human population.

The personal encounters of Hertz (1980:347 - 352) testifies to the significant impact that estrogen and estrogen-mimicking substances has on the reproductive system of both humans and animals.

When two children, a seven year old brother and a five year old sister, presented with bi-lateral modular breast enlargement at the same time, the simultaneous occurrence within the family suggested an exogeneous source of estrogen, particularly since Zondek, Israel, had outbreaks of gynecomastia in a kibbutz attributable to the ingestion of chicken necks that contained residual stilboestrol pellets. An intensive investigation showed that the vitamin supplements that the children were taking contained diethylstilboestrol. The capsules were manufactured in a garage in Philadelphia by a firm that also prepared stilboestrol tablets on the same uncleaned equipment. The Food and Drug Administration (FDA) terminated this practise within days (Hertz, 1980:347-352).

It can thus clearly be seen from the above instances, what a profound effect these estrogen and estrogen-mimicking substances can have on the reproductive systems of both humans and animals.

A more in-depth look at the effect that these estrogen and estrogen-mimicks have on the human population with regard to the reproductive status will follow.

During the 1960's, demographers attributed infertility among couples as being the woman's problem, and not the male's. But the pendulum is swinging in the other direction these days, as the male partners are very much part of the problem. The frequency with which a male factor contributes to a couple's infertility has increased in recent years from about 10 % to 25% (ANON., 1995e:933). There has been an increased frequency of testicular cancer, and boys born with uretral abnormalities, and undescended testicles. Sperm counts have also declined by about a third in the past twenty years - a rate of 2.1%/year, and the quality of sperm, measuring the motility and morphology, has declined as well (Lutz, 1996:12).

In 1992, a Danish team from Copenhagen published an article on the decreasing quality of semen during the past 50 years, from 1938 to 1991 (Carlsen *et al.*, 1992:609-612). On average, the sperm count declined by more than 40%, from 113 million/ml in 1940, to 66 million/ml in 1990. This led to the quote *"Every man in this room is half the man his grandfather was"*. The conclusions that were made by this study indicated that there was a genuine decline in semen quality. As male fertility is to some extent correlated with semen count, the results may indicate a reduction in male fertility. The biological significance of these changes are further emphasised by a concomitant increase in the genital abnormalities (testicular cancer, cryptorchidism and hypospadias), and suggests a growing impact of factors with serious effects on the male reproductive system.

Sperm counts are only one of the many indicators of the male reproductive health system. Testicular cancer in the USA has doubled among white men, and tripled among black men since 1950. Lutz (1996:15), and Sharpe & Skakkebaek (1993:1392-1395) argued that abnormalities like testicular cancer, undescended testicles and urethra abnormalities are all errors that arise during fetal development, and may have a common cause. They suggested that estrogen might be responsible, in part because of the similar abnormalities observed in the sons of the women who were exposed to DES.

There seems to be no conclusive information regarding the mechanism in which the alterations leading to these abnormalities can be made (Raloff, 1994:56-58).

The reproductive health of women are also affected by the estrogen - mimics, in the form of breast cancer, as Wolff *et al.* (1993:648-652) suggested. They concluded that breast cancer was four times more common amongst women who had high blood levels of DDE, a breakdown product of DDT, than women who had low levels. Later that year, a new study indicated that there was no connection between the pesticide and cancer (Krieger *et al.*, 1994:589-599).

In an article of Lee Davis & Bradlow (1995:144-149), the authors spelled out their reasons for suspecting that environmental estrogens, xenoestrogens or foreign estrogens, in particular, contribute to many unexplained cases of breast cancer. In women the incidence of breast cancer in Western Europe and the USA has increased since 1940, and is the most common cancer found in women (Dibb, 1995:27). There is also an increased concern that environmental estrogens might play a role in causing endometriosis, a painful and disabling disease affecting women, which could lead to fertility problems. Formerly a rare disease, it now affects 5 million women in the USA alone (Weiss, 1994:12).

Warhurst (1995a:756) stated that DDT, lindane and atrazine were found to affect the breakdown of estradiol, and thus increases the risk of breast cancer.

It should however be stated that there is no conclusive evidence regarding this matter, and that a lot more research has to be done to make a final conclusion.

Children that were born from women who regularly consumed fish from Lake Michigan which was contaminated with PCB's and other organochlorines, were found to be smaller and had reduced postnatal growth. They had deficiencies in neural development and short term memory which lasted into childhood. The deficiencies were correlated with PCB levels in placental blood and breast milk (ANON., 1993c:22).

The mechanism(s) on how these abnormalities are generated, are unclear. Some researchers conclude the following: during sexual differentiation there are a number of critical periods when the reproductive system is uniquely susceptible to chemically induced perturbations. At these times an inappropriate signal can result in infertility, whereas similarly exposed young

adults are only transiently affected. Many of these abnormalities are not expressed during fetal and neonatal development and only becomes apparent after puberty (Field *et al.*, 1990:47 - 48; Gray, 1992:203). Some scientists believe that estrogen-mimics add to the adult estrogen exposure, and increases the risk of estrogen sensitive breast tumours in women (Dibb, 1995:28), while others say that exposure of the fetus in the womb leads to the most damage in both male and female (Warhurst, 1995a:756).

Exposure to Arochlor 1254, a polychlorinated biphenyl, led to the occurrence of malignant melanoma in two patients (Bahn *et al.*, 1976:450).

Furthermore it was noted that trace quantities of polychlorinated biphenyls (PCB's), polychlorinated dibenzo dioxins (PCDD's) and polychlorinated dibenzofurans (PCDF's), DDT and other insecticides occur commonly in human populations. The major pathway of general population exposure is through foods, inhalation and skin exposure (Reijnders & Brasseur, 1992:166).

Suggestive as all this research is, it is not conclusive, and it could take decades to document any cause-and-effect link between estrogen pollutants and reproductive problems.

In April 1995 the Environmental Protection Agency (EPA) convened a small international scientific conference to discuss a US strategy to better understand the environmental hormones. "The consensus of the workshop was that...[there] was sufficient concern to warrant a concerted research effort..." (Raloff, 1995:46), but still some investigators find the link of these abnormalities and estrogen - mimics unconvincing. In a letter to *Lancet* Thomas (1995:147-151) opposed this matter stating that the supposed linkage was "...dubious and tenuous...". He further commented that "...it is highly doubtful that so-called environmental estrogens have an important role in the search for candidates that might cause insult to the male reproductive

system..." and that testicular cancer, although on the increase, remains very rare.

Karl T. Kelsey of the Havard School of Public Health points out that "although PCB's and DDT metabolites have been shown to have estrogenic activity, other compounds such as birth control pills that have orders of magnitude more activity have not been definitely associated with breast cancer. So it's hard to understand how these compounds could be active when the others are not" (Rennie, 1993:16).

A UK report (Ramsay, 1995:301) published on July 25 1995, points out some of the conflicting variables that need to be taken into consideration when trying to tease out the contribution of environmental estrogens.

- The estrogenic activities of the contaminants used are much lower than those of endogenous estrogens.
- Bans or restrictions on most commercially derived contaminants have diminished their impact on the environment. (Use of organochlorine pesticides has reduced greatly in the past 20 years, at least in the developed world).
- There may be additive or synergistic effects between different categories of xenoestrogens.
- Human beings have always been exposed to phyto-estrogens in plant-derived foods.
- Many people have been exposed to high levels of estrogens in the past 50 years via oral contraceptives and medications.

"...it is currently very difficult to assess whether or not environmental estrogens play a significant role in the reproductive disorders in the general population..." cautions the report.

Stephen Safe of the Texas A & M University, said that the potential risks that the human population endured from the hormone modulating substances was

blown out of proportion. Safe and others make two points in arguing against a demonstrable link between hormone modulating pollutants and human health effects (Stone, 1992:310).

1. They said that most pesticides and environmental estrogens are weak estrogens, and that these substances bind a hundred to thousands of times more weakly to the estrogen receptor than estradiol does, and that this was applicable to both men and women.
2. There are strong doubts that these substances pose a threat to the human population. The doubts of this threat is based on their acid-base argument. Individually a strong base and a strong acid might be corrosive, but when they are mixed, the pair often forms a benign compound. Similarly, Safe and others argue that a sea of natural and synthetic antiestrogens may negate the effect of the environmental estrogens, the net effect being zero.

"The scare was that (these) estrogens were so potent that they were causing an increased incidence of breast cancer" said Michael Gallo of the Robert Wood Johnson Medical Centre at Rutgers University in New Brunswick, New Jersey, on the findings of Wolff (Wolff *et al.*, 1993:648-652), but "the latest paper [(Krieger *et al.*, 1994:589-599)] quieted down those fears" (Stone, 1994:308).

John Gierthy, a toxicologist at the New York State Department of Health's Wadwoth Centre in Albany, worked on the in vitro assay for estrogens, using human breast cancer cell lines, but he is concerned about the relevance of laboratory exposures to the real world. He said that many compounds at high concentrations will increase proliferation of the breast cancer cells, but that it doesn't mean that they will induce an in vivo effect at background levels (Stone, 1994):309.

"We have to admit that we don't know how environmental estrogens affect people, that we're speculating about the effects" says Iowa State historian

Alan Marcus, who has written a history of the politics about DES. Jonathan Li, a reproductive toxicologist at the University of Kansas Medical Centre adds that a lot more research has to be done to establish a relationship between the hormone modulators and disease (Stone, 1994:310).

Some scientists are of the agreement that the threat from the hormone modulators remains a "theoretical" one.

1.3 The role of exogenous estrogens in carcinogenesis.

Quoted from Clark *et al.*, (1980:56) estrogens can possibly play the following role in the carcinogenesis process : "... Chronic exposure of mice and rats to various estrogens resulted in preneoplastic and neoplastic changes in the vagina, uterus, pituitary and mammary gland. Endometrial hyperplasia and cancer occur in women who have been exposed to either endogenous or exogeneous estrogens for prolonged periods of time. These cases include women with ovarian tumours which produce estrogens, women who fail to ovulate and as a result are exposed to estrogen without the normal intervention of the luteal phase of the cycle and women who have taken estrogens for many years because they lack functional ovaries. Exposure of the human fetus to diethylstilbestrol has been associated with the development of vaginal adenosis and clear cell adenocarcinoma in the female offspring. In the studies cited above, the quantities of estrogen administrated were very high and / or the exposure was extended for long periods. Hence, the conclusion that estrogens play some role in carcinogenesis, whether causative or permissive, must be tempered by the realisation that the hormone exposure was non-physiological. Under normal physiological circumstances estrogens may have no carcinogenic potential; however ... it has been suggested that certain estrogens do function in this capacity ..."

In 1971, an epidemiological study was published which indicated that an unusual cluster of a rare form of cancers were found in females aged 14 to 22, all who were related to the in-utero exposure to diethylstilboestrol (DES). It is well documented that exposure of the human foetus to diethylstilboestrol (DES) resulted in vaginal adenosis and clear cell adenocarcinoma in the female offspring (ANON. 1993b:11; ANON. 1995e:933-935; Bilbo *et al.*, 1977:1-8; Faber & Hughes, 1993:35-39; Holden, 1993:1238-1239; Lutz, 1996:12-15; Sharpe & Skakkebaek, 1993:1392-1395; Stillman, 1982:905-921; Stone, 1994:499-500). It is estimated that between 4 and 6 million Americans (mothers, daughters, and sons) have been exposed to DES during pregnancy (Hoover, 1980:353). Males that were exposed to in-utero DES demonstrated a number of teratogenic effects (Hoover, 1980:356). Also they represented with abnormalities of the external genital tract. Their history comprised out of cryptorchidism, hypoplastic testes, epididymal cysts, sperm abnormalities (decreased sperm motility, low sperm counts and abnormal sperm forms). It is often overlooked that the mothers who took the DES during pregnancy had a substantial but short term exposure to exogeneous estrogens. The dose regime during the 1940 - 1950's was 10 to 12 grams of DES that was to be administered during pregnancy. Further studies also indicated that the DES-exposed women were more likely to develop ovarian and uterine cervix cancer than women who weren't exposed (Hoover, 1980:357).

Conjugated estrogens that were used in the late 1970's for the treatment of climacteric disorders was indicated to have been associated with a relative high risk of endometrial cancer (Hoover, 1980:357).

DES and estrone are considered to have specific cancer forming "properties". In contrast to this, estriol has been proposed to be a protective agent against the development of neoplasia (Clark *et al.*, 1980:56).

The situation for oral contraceptives is somewhat different from those described above. The sequential use of oral contraceptives has been related to an increased risk of endometrial cancer in young women and the prolonged use of it has been shown to be linked to benign neoplastic liver tumours. Suspicions have also been raised with respect to oral contraceptive use and the increased risk of breast and cervix cancer, with respect to a specific group of women, particularly the high risk women (Hoover, 1980:373).

Chapter

2

New insights regarding the occurrence of estrogen and estrogen-mimics in the environment

In a recent Environmental Data Services (ENDS) report 249 (ANON., 1995b:4-5), it was commented that the search for estrogenic chemicals in sewage effluent, a three year project of £ 250 000, funded by the Environmental Agency, has reached a critical stage, as the majority of the estrogenic activity was tracked down to one small effluent fraction that does not contain known estrogens such as alkylphenols. Research was undertaken to identify the mystery compound(s).

Of the compounds found in the same effluent were detergent compounds alkylphenol ethoxylates (APE) and some of their breakdown products, alkylphenols. These levels were particularly high in wool scouring effluents, and had severe effects in the river Aire in Yorkshire. Once again the wildlife was affected as caged fish displayed retarded testicular development likely to inhibit successful reproduction. After a Glaxo test (see section on *Screening and testing of environmental hormone disrupters [Chapter 4]*) it was concluded that the volatile and particulate fractions of sewage has no estrogenic activity and neither did non polar organic or inorganic compounds. From the liquid chromatography it was revealed that the vast majority of the activity was concentrated in a single fraction, which eluted from the chromatography column after 25 minutes. It was this fraction that accounted for at least 80% of the estrogenic activity in all of the effluents that were taken. APE's and alkylphenols were not found in this fraction, suggesting that if they were involved, the yet to be identified intermediates which caused the activity has still to be discovered.

In a later ENDS report 261 (ANON., 1996c:8-9) these substances were identified as being mainly natural estrogens : oestrone and 17β -oestradiol and the synthetic estrogen ethinylestradiol. Alkylphenols were also found to be present, but at such low levels that it did not register in the estrogenic assay or cause effects on fish.

The researchers believe that human excretion products were the most likely source, but this was not proven. Humans and animals excrete estrogen in inactive forms which appear to be converted back to active hormones during sewage treatment. Pearce (1996:7) collaborated further on this new discovery and concluded that oral contraceptives are not to be blamed for the gender bending effects seen in fish living downstream from sewage treatment works, research has identified these substances being natural estrogens excreted by women whether or not they were taking the Pill. and the synthetic estrogen, ethinylestradiol.

Johnson (1996:331A) stated that synthetic estrogens that are individually weak have up to a 1000 times more estrogenic potency when combined. Substances like dieldrin, toxaphene and endosulfan has individual potencies of 1/10000 th that of the natural estrogen, 17β -estradiol.

The Scottish Environmental Protection Agency (SEPA) has found potentially harmful concentrations of two hormone disrupting chemicals in 15% of the samples taken in a comprehensive survey of major industrial and municipal sewage discharges entering surface waters throughout Scotland. The SEPA initiated a study to determine levels of alkylphenol ethoxylates (APE's) and phthalates - two classes widely associated with estrogenic effects (ANON. 1996c:8-9).

Discharges from nine sewage treatment works (STW's), two sea outfalls, and one industrial effluent treatment plant were found to contain levels of nonylphenol which results from nonylphenol ethoxylates that exceed the 1-2 µg/l level (ANON., 1996c:8-9).

For the 15 phthalate esters sampled, one STW and one sea outfall contained levels that exceeded the 1-2 µg/l level. SEPA is however adopting a precautionary approach and is actively discussing to substitute these compounds with safer ones. It is hoped that these substances will be phased out before the UK Government's target date of 2000 (ANON., 1996c:8-9).

Chapter

3

Growing international concern about estrogen and estrogen-mimics

A recent ENDS report 249 (ANON., 1995b:4-5) commented on calls that were made for new legal controls of the estrogen and estrogen-mimics, as these substances has an effect on human and animal health.

Johnson (1996:331A) reported on a consensus statement from 18 scientists concerning the significance of hormone-disrupting chemicals. It urged that greater research, communication and other actions had to be undertaken to address the threat that these chemicals pose. Furthermore the public should be made aware, manufacturers should assure the consumers that their products are safe and demonstrate that these chemicals pose no developmental health hazards.

In an *Environmental Science and Technology News* article (ANON., 1996b:476) it was reported that the US Government was funding some 400 research projects that examine the effects of chemicals that disrupt the endocrine systems of humans and wildlife. A 26 member federal panel organised by the White House National Science and Technology Council intends to make a searchable database of endocrine - disrupter research and combine it with information on similar public and private research in the USA and Europe. The database is intended to help scientists co-ordinate research, see where gaps lie, and may serve as a model for other complex research projects.

Warhurst (1997) reported on a powerful new alliance fight against gender benders stating that a recent letter to the *Times* (31.07.1995) the following was

published of a group of experts and campaigners towards the demand to phase out chemicals that disrupt the reproductive system :

"If ever there was a case for applying the 'precautionary principle' (i.e. it's better to be safe than sorry), and phasing out the groups of chemicals that are the principal subjects, then surely this must be it."

They concluded saying :

"We have enough experience of the real human health, environmental and financial costs of the past misjudgements about asbestos, PCB's and ozone depletion to act urgently on this serious threat. Inevitably there will be some short term difficulties for some companies. However, when faced with a choice, the consequences of one which may be difficult for a few and the other unthinkable for the many, then the route to be taken is obvious."

Dr Theo Colborn (1996), Chief Scientist of the World Wildlife Fund, gave a speech on the 3rd of October 1996 on Toxic chemicals also stating that more than 100 nations has been called for negotiations of an international agreement to reduce or eliminate persistent organic pollutants (POP's), some of which are endocrine disrupters. She also stated that the US Congress passed several mandates acknowledging this problem.

Chapter

4

**Screening and testing of environmental
hormone disrupters**

Researchers with a broad range of opinions on endocrine disrupters, all agree that developing a strategy to screen and test for such chemicals is an essential element in deciding what to do about them.

Lynn R. Goldman, assistant administrator of the Environmental Protection Agency's (EPA) office of Prevention, Pesticides, and Toxic Substances, commented on this subject as follows : " We do not currently know the extent to which problems from endocrine disrupters exist ... But in view of existing data, endocrine disrupters warrant priority attention now " (Hileman, 1996:27).

A workshop held in May 1996, sponsored by the EPA, focused on how to test for these environmental disrupters. During the course of this gathering, several participants noted that chemicals are not tested for endocrine disruption, in fact, except for pesticides, most chemicals were not tested for any biological endpoint. At this stage, a screening strategy is one of a theoretical kind, as there are no existing method that has the ability to detect false positive or false negative results thus necessitating a mixed in vitro / in vivo strategy (Hileman, 1996:27).

Throughout this discussion, it was emphasised that the need existed for co-operation with groups in both the public and private sector that are working on endocrine disruptive issues. The suggestion was made that the first two steps would be to develop processes for prioritising chemicals to be screened and for completing the needed testing. The participants agreed that the tests should be limited to receptor-mediated outcomes, and that tests would not be

developed for the chemical's ability to cause indirect effects, such as effects on the liver that result in hormone disruption (Hileman, 1996:27). The environmental estrogens received special interest during this gathering.

In a recent article of Patlak (1996:540-544), the EPA got a tight timetable for action to develop a screening method to test common chemicals for endocrine disruptive effects, and the program must be implemented just one year thereafter. The EPA already began to decide which of the 80000 commercial chemicals should be tested for hormonal activity, and the number has already shrunk to 17000 common chemicals that are in commerce at high volume. Lynn Goldman plans to further shrink this number by focusing on chemicals found in drinking water, food, ambient air, or household products. The EPA researchers are also scrutinising the three dimensional structures of known endocrine disrupters to look for similar traits that can be used to predict which groups of chemicals are likely to have hormonal effects.

These chemicals pose a new set of challenges in that the effects can vary, depending on which tissues are tested, when during an organism's development the compound is administered, and whether other chemicals are present. Some effects may not surface for more than 25 years, and, unlike most toxic substances, the greatest effect of these substances are seen at the smallest doses tested.

A recently developed test, using an ingenious yeast estrogen assay was developed by **Glaxo**. The test uses a genetically engineered yeast strain expressing the human estrogen receptor. When the molecule binds to the receptor, a receptor gene is switched on which produces an enzyme. This in turn triggers a colour change in the substrate allowing fractions containing estrogenic substances to be identified rapidly (ANON. 1995b:5).

Of the existing methods of testing for estrogenic activity, the administration of a chemical to an animal with immature or removed ovaries (and thus without

their own source of estrogen) over several days, to test its estrogenic property exists. The uteri of these animals are then compared with those of control animals, to see if any uterine growth was prompted (Patlak, 1996:541).

An easier and less expensive method, is the ***E - screen***. This method measures how much of a compound prompts cultured breast cancer cells to multiply. The cancer cells that harbour estrogen-receptors, grow only if estrogen or estrogen-mimics are added. The amount of cell proliferation is then compared to the cell proliferation of that prompted by estradiol over the same period of time (6 days), to determine the compound's estrogenic rating (Patlak, 1996:541).

Other tests used to measure estrogenic effects, measure the amount of gene activity triggered by chemicals latching on to estrogen receptors in the cell nucleus (Pasqualini *et al.*, 1988:541).

None of the above mentioned tests detect the effects of a compound on the developing fetus. The requirements for this testing takes long and is very costly.

Daniel Sheeman, of the U.S. Food and Drug Administration's Centre for Toxicological Research has developed an ***inexpensive and short-term animal test*** that shows promise for quantifying the effects of an environmental estrogen compound during prenatal development. The test measures what level of environmental estrogens administered to pregnant mice prompt uterine cells in their female foetuses to multiply. This assay can also be used to assess the effects of the environmental estrogens stored in fat tissue and released into circulation during pregnancy, by administering the estrogenic compounds before pregnancy. To determine the effects released into breast milk, the amount of uterine proliferation seen in unexposed mouse

pups who are nursed by mothers prenatally exposed to environmental estrogens are measured (Patlak, 1996:544).

Chapter**5****The female hormone estrogen**

Estrogens are hormones which stimulate growth and development of the secondary sex characteristics and organs of the female animal, and thus contributes an important component of the female physiology. Yet it is well known that estrogens can be dangerous substances if handled improperly, or under certain abnormal patho-physiological states e.g. liver cirrhosis (Clark *et al.*, 1980: 53).

Estrogen has a wide variety of uses in the pharmaceutical industry, ranging from the usage as a contraceptive agent (the Pill) or for the alleviation of menopausal syndromes. Furthermore it is known that estrogen therapy is very beneficial in the treatment of acne, as well as in the treatment of menstrual irregularities in young women. Estrogen also is known for its ability to enhance bone resorption and thus finds its applicability in preventing osteoporosis, the brittle bone disease.

5.1 Estrogen as a contraceptive agent.

The combined oral contraceptives contain an estrogen (either 17- α Ethinyl estradiol or mestranol) and a progestin. This combination makes the Pill the most reliable method of contraception. The lower pregnancy rate is due to the estrogen action on the hypothalamic-pituitary axis. Follicle stimulating hormone (FSH) secretion is reduced, resulting in the failure of the ovarian follicles to mature. The estrogen also acts at the endometrium to prevent irregular shedding of cells which leads to bleeding between periods (Einsensfeld *et al.*, 1980:90).

Rare but serious side effects associated with the usage of the Pill have been related to the presence of estrogen. Estrogens may contribute to the increased incidence of thrombosis, heart attacks, hypertension, gallbladder disease and liver tumours. The incidence of heart attacks are mostly marked in women who use the Pill in combination with smoking cigarettes, and who is over 40 years of age (Einsensfeld *et al.*, 1980:91).

Estrogens are also used in other clinical situations. Examples of these are during or after menopause, for the treatment of osteoporosis, acne treatment in women, as well as for the treatment of women who suffer from impaired menstrual cycles, due to not ovulating.

The newer combined oral contraceptives contain lower amounts of estrogen, ethinyl estradiol, and of the progestin. Decreasing the amount of ethinylestradiol to about 30 µg seem to retain the contraceptive effectiveness (Einsensfeld *et al.*, 1980:92).

Table 5.1 gives a detailed list of products on the South African market that contains estrogen and that are used as either oral contraceptives or for the treatment of menopausal disorders.

Table 5.1 : Estrogen products used in oral contraceptives and in menopausal disorder treatments (just the estrogen content is noted) .

Contraceptive use

Product	Manufacturer	Estrogen component
Biphasil ®	Akromed	Ethinyl oestradiol 50 µg
Brevinor ®	Roche	Ethinylestradiol 35µg
Demulen ®	Searle Goldscript	Ethinyl oestradiol 50µg
Diane 35 ®	Schering	Etinylestradiol 35 µg
Femodene ED ®	Schering	Etinylestradiol 30 µg
Logynon ED ®	Schering	Ethinyl estradiol 30 µg & 40 µg
Marvelon 150/30 ®	Donmed	Ethinylestradiol 30 µg
Mercilon ®	Donmed	Etinylestradiol 20 µg
Minovlar ED ®	Schering	Etinylestradiol 50 µg
Minulette ®	Akromed	Ethinyl oestradiol 30 µg
Nordette ®	Akromed	Etinyl oestradiol 30 µg
Nordiol ®	Akromed	Etinyl oestradiol 50 µg
Norinyl 1/28 ®	Roche	Mestranol 50 µg
Normovlar ED ®	Schering	Etinylestradiol 50 µg
Ovostat - 28 ®	Donmed	Ethinyl oestradiol 50 µg
Ovral ®	Akromed	Etinyl oestradiol 50 µg
Tri - Minulette ®	Akromed	Ethinyl oestradiol 30 µg & 40µg
Triphasil ®	Akromed	Etinylestradiol 30 µg & 40 µg
Trinovum ®	Janssen	Ethinyl oestradiol 35 µg
Triodene ®	Schering	Etinylestradiol 30 µg & 40 µg

Table 5.1 (continued) : Estrogen products used in oral contraceptives and in menopausal disorder treatments (just the estrogen content is noted) .

Menopausal disorders

Product	Manufacturer	Estrogen component
Estinyl ®	Schering - Plough	Ethinyl oestradiol 0.02 mg & 0.05 mg
Estraderm TTS 25, 50, 100 ®	Ciba	Oestradiol 2 mg, 4 mg & 8 mg
Estrofem ®	Novo Nordisk	Estradiol 2 mg
Evovel ®	Janssen	β - estradiol 3.2 mg
Honvan ®	Noristan	Diethylstilboestrol diphosphate 50 mg/ml
Menoflush ®	Pharm Ent	Ethinyl oestradiol 0.02 mg
Oestradiol ®	Donmed	Oestradiol 20 mg
Premarin ®	Akromed	Conjugated oestrogen lactone 200 mg, 0.625 mg, 1.25 mg
Primogyn Depot ®	Schering	Estradiol valerate 10 mg/ml
Progynova ®	Schering	Oestradiol valerate 2 mg
Synapause ®	Donmed	Oestriol succinate 2 mg
Vagifem ®	Novo - Nordisk	Estradiol 25 µg

Table 5.1 (continued) : Estrogen products used in oral contraceptives and in menopausal disorder treatments (just the estrogen content is noted) .

Menopausal disorders : Combinations

Product	Manufacturer	Estrogen component
Climen ®	Schering	Estradiol-17-valerate 2 mg
Divana ®	Continental Ethicals	Oestradiol valerate 2 mg
Estracombi TTS ®	Ciba	4x Estaderm TTS 50 (Oestradiol 4 mg) 4x Estragest TTS (Oestradiol 10 mg)
Mixogen ®	Donmed	Estradiol benzoate 1 mg Estradiol phenylpropionate 4 mg
Postoval ®	Akromed	Oestradiol valerate 2 mg & 4 mg
Prempak N ®	Akromed	Conjugated oestrogen 0.625 mg & 1.25 mg
Trisequens ®	Novo - Nordisk	Estradiol 2 mg & 1 mg

* Compiled from Anon., 1995f:205-210 & Anon., 1997b:203-215.

Chapter

6

***Obtaining information regarding estrogen
and estrogen-mimic locations in the
South African environment***

An intensive investigation was made (a literature study of where estrogen and estrogen-mimics were found in the South African water environment and a series of telephone calls, which extended over a period of approximately 2½ months) regarding the possible identification and location of estrogen and estrogen-mimicking substances.

Institutions like Government departments, research facilities, chemical manufacturing industries and educational institutions were contacted per telephone (refer to appendix 4 for a detailed directory of the institutions contacted). A fax explaining the purpose of the study, and a list of estrogen and estrogen mimicking substances were sent to the various institutions with the request for assistance and comment (refer to appendix 5).

Institutions who received faxes are listed in appendix 4a, and appendix 4b gives a detailed directory of people contacted by phone.

Chapter

7

Analytical methods for the determination of estrogen and estrogen-mimics in water.

7.1 *The collection of water samples.*

The four basic factors that affect the quality of environmental data such as the analysis of water samples are sample collection, preservation, analysis and data recording on equipment like, in the case of this study, HPLC and GC. Improper action in any of the steps mentioned, will result in poor data judgements, and inaccurate results.

Most definitions of water quality are user related. Most users produce waters containing pollutants which has an impact on the environment in different ways.

Sampling is thus the key element in the monitoring of the water quality, and should be performed properly to ensure valid data. The sampling sites should be carefully selected to ensure that the *right sample* is taken at the *right place* at the *right time* and in the *right way*.

During the course of this investigation a variety of water samples - such as river and dam water and sewage and industrial effluent were tested for the occurrence of estrogen and estrogen - mimics.

The procedures that were followed to ensure that the best representative sample was collected were as follows :

- 2,5 L Amber glass bottles were prerinsed with distilled water and left to dry (glass bottles were chosen because most of the samples contained organic compounds).
- The bottles were then closed and clearly labelled, containing the name of the sampling area.

- The bottles were transported to the sampling area, where the sampling took place.
- The sampling was done as follows :

in the case where **industrial effluent** was taken, the sampling was done by lowering a bucket into the manhole at the discharge site. The water was then transferred into the supplied bottles. This was done either before the effluent entered the river or at intervals 0, 10 and 24 km from the Barrage

dam and river samples were sampled by lowering the bucket approximately 40cm under the water and allowing it to fill. The water content was then transferred to the bottles

the **drinking water samples** were taken directly from the water taps in the following areas of investigation : Windhoek, Pretoria, Johannesburg and Vereeniging

in the **sewage effluent** case, a very sensitive and selective choice was made regarding the type of sample taken. The choice was made to sample only the effluent that went through the cleaning process, thus preventing the troublesome and inconvenient analysis of raw sewage. The sewage plants that were investigated during this study were the following : Durban, Vereeniging, Potchefstroom, Cape Town, Memel, Windhoek Municipality, Bloemspruit Works (Bloemfontein), the Pietersburg Sewage Works and the Northern and Southern Works of both Johannesburg and Pretoria.

Samples were analysed as soon as possible after the sampling took place, and was thus in some cases only preserved with a few drops of analytical grade chloroform. All the samples were refrigerated during storage.

7.2 Selection of sampling points and the reasoning behind it.

In the paragraphs that follow, the selection and reasoning behind the taking of samples at different locations will briefly be discussed.

During the literature study a list of all the estrogen and estrogen - mimics mentioned in the literature was compiled. A collection of 142 substances were gathered (refer to appendix 1) and classified into similar groups regarding their uses (refer to appendix 2).

This list was reduced to those substances that were most likely to be found in the South African water environment. The following elimination procedures were applied to reduce the list of substances :

- *Personal visits* were undertaken to institutions such as Karbochem, Sasolburg, and the Institute for Water Quality Studies (Pretoria) for guidance as to which of these substances were most likely to be found in South Africa, and especially in the water environment.
- *Personal communications* were made by telephone followed up by a fax to various institutions over a period of 2½ months to determine which of these substances were already found in South Africa, what their specific location was as well as at what concentration levels they were found.
- An investigation of the *import quantities* of some of the estrogen and estrogen - mimics was made but with little success because of lack of co-operation (refer to appendix 6). This data would have been useful as to see which of the substances are used more than others in terms of import and export values.
- A data search through *Chemdata* was undertaken to determine which of these substances are manufactured locally (refer to appendix 7).

- Elimination by means of the *chemical characteristics* (water solubility & melting points) [refer to appendix 8] *and some toxicological data* (LD_{50} & LC_{50} values) [refer to appendix 9].
- Eliminating from the list substances that were too *vaguely described* such as “some PCB’s” and “organochlorinated hydrocarbons” which covered a too wide spectrum of substances as well as substances that were highly unlikely to pollute the water environment in a harmful manner : eg. “plastic containers”, “boron”, “musk ketone and scent”, “Clone ID5”, “carboxylic acid” and “chicken manure”.
- Eliminating from the list those substances that were *not able to be determined and analysed in South Africa* due to reasons such as inadequate methods or equipment as well as due to the high cost involved in analysing these substances - as was the case with the dioxin determination.

The above resulted in a list of estrogen and estrogen - mimics that could be tested and analysed for at specifically allocated areas in South Africa (refer to appendix 9).

The reasoning behind the locations selected were as follows :

- The Vaalriver (monitored at Vereeniging) was sampled at varying sites taken from the Barrage as to the huge amount of factories neighbouring or situated around it (e.g. Sasol, Karbochem, Orchem, AECI Industrial Chemicals and Polifin). Taking the samples at intervals 0, 10 and 24 km from the Barrage made it possible to assess what the concentration levels were at different distances from the Barrage).

- The effluent of Chemical manufacturing companies such as AECI Industrial Chemicals, Sasol, Sanachem and Polifin were inspected because of their local manufacturing of estrogen - mimics such as nonylphenol (Sasol), DDT, DDE, DDD, methoxychlor, toxaphene, endosulfan, malathion, chlordane, dicofol which could thus lead to unintentional spillage into the water environment.
- The PWV's largest sewage effluent sites situated near Rand Water's covering sites (Vereenigning and Johannesburg Northern area) were investigated as well as others (Bloemfontein, Durban, Cape Town, Pretoria Northern and Southern area, Johannesburg Southern area and Potchefstroom) as well as specially predicated areas such as Memel and Pietersburg - where there were reported cases of a shift in the sex ratio of fish near the sewage plant - and Windhoek where they use reversed osmosis in order to recycle the water, thus having the biggest chance of concentrating estrogen levels in Southern Africa.

For a summary indicating which estrogen and estrogen -mimics are to be tested for in the South African water environment refer to appendix 9.

7.3 The determination of estrogen in water.

It should be noted that at the time of this investigation *no* references could be found in the literature with regard to the detection of estrogen in water. Several attempts were made from various sources, but none could be found. The only references that existed with regard to estrogen detection was that of the detection of estrogens in urine and plasma, and thus found no applicability in this investigation.

This part of the analysis was done in part by the Potchefstroom University and The Jockey Club of Southern Africa.

The Potchefstroom University did the extraction, while the Jockey Club assisted with the detection of the estrogen in sewage effluent by means of Gas Chromatography / Mass spectrometry (GC-MS).

The GC-MS analysis was performed by the Jockey Club of Southern Africa, which is an accredited analytical facility. No details of the method was supplied.

Samples were extracted by means of solid phase extraction (SPE) followed by a further clean - up procedure utilising Immuno affinity column chromatography (IAC).

IAC utilises an antibody to selectively retain a certain compound, thus eliminating interfering compounds that might be co - extracted when using SPE alone.

All chemicals that were used were supplied by Merck (E, Merck, Midrand, SA) and Saarchem (Muldersdrift, SA) and were of analytical grade.

The methanol, chloroform, dichloromethane and acetonitrile used were of HPLC analytical grade (Hi Persolv, BDH, Poole, England).

Sample extraction :

Solid phase extraction

- SPE columns (3cc / 500 mg Bond Elut ®, C₁₈ columns, Varian, Harbor City, CA) were conditioned with 6 ml of methanol, followed by 6 ml of water.
- To the 1l sample, 2 ml of orthophosphoric acid was added, and was put through a 0.7 µm glass fibre filter (Whatman GF/F, Whatman International Ltd., Maidstone, England)
- The 1l sample was then put through the columns by means of a large volume extraction tube under vacuum.
- The SPE columns were then rinsed with 6 ml of water.
- Columns were dried by suction.
- Elution was done with 4 ml of methanol.
- The eluant was placed in a waterbath at 47 °C, and dried with a stream of nitrogen gas.
- 2 ml of washing solution was then added and put through the IAC.

Immuno affinity column extraction procedure (IAC)

The following solutions were made prior to immuno affinity extraction :

PBS solution :

NaCl	9 g/l
Na ₂ HPO ₄ ·2H ₂ O	7.9 g/l
KH ₂ PO ₄	0.75 g/l
NaN ₃	1 g/l

The pH was set to 7.5 with NaOH or H₃PO₄

Washing solution:

MeOH / distilled H₂O v/v 10/90

Elution solution :

MeOH / distilled H₂O v/v 80/20

Special immunoaffinity gels (ethinylestradiol and diethylstilboestrol) were ordered from the Laboratory of Hormonology, Rue du Point du Jour 8, B-6900, Marloie, Belgium.

Their specifications were as follows :

Specificity :

	<i>Ethinylestradiol</i>	<i>DES</i>
Antiserum	Anti-ethinylestradiol	Anti-diethylstilbestrol
Animal number	Rabbit M111 - 07.07.88	Rabbit 99 - 10.12.75
Gel matrix	Sepharose® CNBr4B	Sepharose® CNBr4B
Capacity determined with tritiated tracer	> 50 ng/ml of gel	> 50 ng/ml of gel

Cross reaction of the antibody in RIA system (ethinylestradiol) :

- ethinylestradiol	100 %
- norethindrone	10 %
- 17 β estradiol	0 %
- estrone	0 %

Cross reaction of the antibody in RIA system (diethylstilboestrol) :

- trans - diethylstilboestrol (DES)	100 %
- trans - DES di-n-propionate	9.7 %

- hexestrol	3.3 %
- dienestrol	1 %
- trans - DES -dimethylether	0.4 %
- benzeestrol	0.1 %

Immuno affinity column extraction (IAC) :

- 1 ml of gel of ethinylestradiol and the internal standard diethylstilboestrol (DES) was transferred to a supplied column.
- The column with gels were then washed with 2 x 5 ml of PBS 0.05 M (pH = 7.5) solution.
- The gel was equilibrated with 3 x 3 ml of distilled water.
- The sample was applied.
- The columns were then washed with 3 x 3 ml of washing solution.
- The gel was eluted with 3 ml of elution solution.
- The eluate was dried in a waterbath at 47 °C with a stream of nitrogen gas.
- 50 µl of methanol was added, vortexed, and transferred to a vial for analysis.

The columns with gel were washed with 1 ml of elution solution, regenerated with 2 x 5 ml of PBS solution and stored at 4° C in a PBS solution for re-use.

7.4 The determination of alkylphenolic compounds such as nonylphenol in water.

The analyses were performed at the CSIR's Food Science and Technology division in Pretoria which is an accredited analytical facility. They used proven methods of extraction and the analysis was performed by means of HPLC on a reversed phase column with fluorescence detection. Unfortunately exact details in the methodology were not available to us.

7.5 The determination of organochlorine pesticides in water.

The analyses were performed by the General Chemistry division SABS, which is also an accredited analytical facility.

Pesticides analysed in this category were : *DDT total* (all the o'p' and p'p' forms of DDD, DDE and DDT), *methoxychlor*, *endosulfan* (α and β isomers), *chlordane* and *dicofol*.

Conditions (as stipulated for chlordane) :

- **Instrument** : Hewlett Packard Series 6890
- **Columns** : Column 1 : HP-5 5 % Phenyl methyl siloxane, 0.25 μm ,
30m x 320 μm (Hewlett Packard, Palo Alto, CA)
Column 2 : HP-1 Methyl siloxane, 2.65 μm ,
30.0m x 530 μm (Hewlett Packard, Palo Alto, CA)
- **Flow rate** : Column 1 : 1.7 ml/min, column 2 : 4.0 ml/min
- **Volume injected** : 1.0 μl
- **Runtime** : 30 minutes
- **Column temperature** : Column 1 & 2 : ambient
- **Detector** : Front : ECD with nitrogen gas, Back : NPD with nitrogen gas

Conditions (as stipulated for α and β endosulfan) :

- **Instrument :** Hewlett Packard Series 6890
- **Column :** Column 1 : HP-5 5% Phenyl methyl siloxane 0.25 μ m,
30.0m x 25 μ m (Hewlett Packard, Palo Alto, CA)
- **Flow rate :** 0.8 ml/min
- **Volume injected :** 1.0 μ l
- **Runtime :** 29 minutes
- **Column temperature :** not indicated
- **Detector :** MSD Transfer Line Heater
- **Retention time :** α endosulfan : 20.49 min and β endosulfan : 22.13 min

Conditions (as stipulated for DDT total and methoxychlor) :

- **Instrument :** Hewlett Packard Series 6890
- **Column :** Column 1 : HP-5 5% Phenyl methyl siloxane 0.25 μ m,
30.0m x 25 μ m (Hewlett Packard, Palo Alto, CA)
- **Flow rate :** 0.8 ml/min
- **Volume injected :** 1.0 μ l
- **Runtime :** 29 minutes
- **Column temperature :** not indicated
- **Detector :** MSD Transfer Line Heater
- **Retention time :** p'p' DDE : 21.08 minutes, p'p' DDD : 22.39 minutes,
o'p' DDT : 22.50 minutes, p'p' DDT : 23.80 minutes and
methoxychlor : 26.74 minutes

Method used for the analysis :

Sample extraction :

1. Each sample was well shaken before a volume of 500ml was extracted in duplicate.
2. The pesticides were extracted according to the Association of Official Analytical Chemists' method No. 990.06 (Sawyer *et al.*, 1995:13 - 17).

Final analyses were carried out by gas chromatography employing electron capture, flame photometric, nitrogen / phosphorus selective, or by mass selective detection.

3. Recovery determinations were carried out by adding known amounts of the relevant active ingredients to portions of tap water and analysing these concurrently with the samples.

Recovery determinations done at 1µg/l gave the following mean values :

<i>α Endosulfan</i>	120 %
<i>β Endosulfan</i>	113 %
<i>Lindane (gamma BHC)</i>	91 %
<i>Chlordane</i>	90 %
<i>Methoxychlor</i>	133 %
<i>p'p' DDE</i>	128 %
<i>p'p' DDD</i>	134 %
<i>o'p' DDT</i>	126 %
<i>p'p' DDT</i>	129 %

Lowest limit of quantitation was 1µg/l.

7.6 The determination of organophosphate pesticides in water.

This part of the analyses were also performed by the General Chemistry division SABS.

The pesticide analysed in this category was *malathion*.

Conditions :

- **Instrument :** Hewlett Packard Series 6890
- **Column :** Column 1 : HP-5 5% Phenyl methyl siloxane 0.25 μ m,
30.0m x 25 μ m (Hewlett Packard, Palo Alto, CA)
- **Flow rate :** 0.8 ml/min
- **Volume injected :** 1.0 μ l
- **Runtime :** 29 minutes
- **Column temperature :** ambient
- **Detector :** MSD Transfer Line Heater
- **Retention time :** 18.69 min

Method used for the analysis :

Sample extraction :

1. The sample was well shaken before a volume of 500ml was extracted in duplicate.
2. The pesticides were extracted according to the Association of Official Analytical Chemists' method No. 990.06 (Sawyer *et al.*, 1995:13 - 17).

Final analyses were carried out by gas chromatography employing a mass selective detector.

Final analyses were carried out by gas chromatography employing a mass selective detector.

3. Recovery determinations were carried out by adding known amounts of the relevant active ingredients to portions of tap water and analysing these concurrently with the samples.

The **recovery determination** done at a level of 1 µg/l gave a mean value of 132 % for malathion.

Lowest limit of quantitation was 1µg/l.

7.7 The determination of polychlorinated biphenyls (PCB's) in water.

This part of the analyses were also performed by the General Chemistry division SABS.

The polychlorinated biphenyls (PCB's) that were investigated were Arochlor 1254 and Arochlor 1260.

Conditions :

- **Instrument :** Hewlett Packard Series 6890
- **Columns used :** Column 1 : HP-5 5 % Phenyl methyl siloxane, 0.25 μm ,
30m x 320 μm (Hewlett Packard, Palo Alto,
CA)
Column 2 : HP-1 Methyl siloxane, 2.65 μm ,
30.0m x 530 μm (Hewlett Packard, Palo Alto,
CA)
- **Flow rate :** Column 1 : 1.7 ml/min, column 2 : 4.0 ml/min
- **Volume injected :** 1.0 μl
- **Runtime :** 30 minutes
- **Column temperature :** Column 1 & 2 : ambient
- **Detector :** Front : ECD with nitrogen gas, Back : NPD with nitrogen gas

Method used for the analysis :

Sample extraction :

1. The sample was well shaken before a volume of 500ml was extracted in duplicate.
2. The pesticides were extracted according to the Association of Official Analytical Chemists' method No. 990.06 (Sawyer *et al.*, 1995:13 - 17).

Final analyses were carried out by gas chromatography employing electron capture and nitrogen / phosphorus selective detection.

3. Recovery determinations were carried out by adding known amounts of the relevant active ingredients to portions of tap water and analysing these concurrently with the samples.

Recovery determinations done at a level of 1 µg/l gave the following mean values :

<i>Arochlor 1245</i>	92 %
<i>Arochlor 1260</i>	72 %

Lowest limit of quantitation was 1µg/l.

7.8 The determination of triazine herbicides such as atrazine in water.

7.8.1 Atrazine determination in dam water.

All chemicals that were used were supplied by Merck (E, Merck, Midrand, SA) and Saarchem (Muldersdrift, SA) and were of analytical grade.

The methanol, chloroform, dichloromethane and acetonitrile used were of HPLC analytical grade (Hi Persolv, BDH, Poole, England).

Description :

HPLC in combination with liquid-liquid extraction (LLE) was used.

It should be mentioned that Solid phase extraction (SPE) was also undertaken prior to undertaking LLE, but wasn't successful because the columns blocked with the particles in tap water.

Doing an atrazine analysis, no filtration prior to the extraction procedure must be done due to the fact that atrazine adheres to dust and sediment particles in water. Filtration would thus result in obtaining incorrect values leading to incorrect assumptions.

HPLC conditions :

- **Instrument :** Hewlett Packard Series 1050 HPLC equipped with an autosampler, gradient pump, UV detector and HP Chemstation data handling and control system
- **Column :** Hypersil ODS, 250 x 4.6 mm , 5 μ m (Phenomenex, Torrance, CA)
- **Internal standard :** Terbutylazine (TBA)
- **Flow rate :** 1 ml/min
- **Volume injected :** 100 μ l
- **Runtime :** 15 min
- **Column temperature :** 40 °C
- **Detector :** UV at 220nm
- **Retention times :** Atrazine (5.96 minutes) and terbutylazine (8.99 minutes)
- **Mobile phase :** (50 :50) Acetonitrile : 0.01 M ammoniumdihydrogen-orthophosphate.
Final pH 6.5

Extraction procedure :

- 500ml of the sample was accurately measured off.
- 2ml of a 0.50 mg/l solution of TBA (internal standard) and 5 ml of 6N ammonium hydroxide solution were added to the sample.
- The sample was then shaken for 2-3 minutes, and extracted with 3x 30ml dichloromethane.
- It was then centrifuged at 3000 rpm for 5 minutes.
- The top layer was then carefully removed by suction with a Pasteur pipette and discarded.
- The sample was then placed in a waterbath at 47 °C and dried under a stream of nitrogen gas.

- The sample was then placed in a waterbath at 47 °C and dried under a stream of nitrogen gas.
- After the drying procedure 5ml of mobile phase was added to the sample and placed in an ultrasonic waterbath to disperse.
- The sample was then placed in a labelled vial and injected in the HPLC.

Method validation parameters

- **Linearity and range :**

The linearity was determined by preparing a set of 5 standards with concentrations of 0.25, 0.5, 1.0, 2.5, and 5µg/l in duplicate. The standard solutions were analysed by HPLC, and the results were used to construct a standard curve.

The results were as follows :

Regression output :

Constant :	+0.0351
X - Coefficient :	0.8186
Regression coefficient :	0.9975

- **Accuracy and precision :**

Accuracy and precision was determined by preparing 5 samples of 1.0 µg/l and 5 samples of 5.0 µg/l. These were then analysed and the % recovery and % RSD calculated. The accuracy (% recovery) was found to be 105.05% and the precision (% RSD) was 6.58%.

- **Limit of quantitation and limit of detection**

The *limit of quantitation* was 0.25 µg/l, with a % RSD of 15.08.

The limit of detection (peak three times the height of the baseline noise) was 0.05 µg/l.

- **Extraction efficiency**

The extraction efficiency was determined by comparing the peak areas obtained from the extracted samples to the areas of standards prepared by making the appropriate dilutions in the mobile phase.

The *extraction efficiency* for :

1.0 µg/l atrazine was 78.22 %

5.0 µg/l atrazine was 83.37 %

1.0 µg/l terbutylazine was 75.27 %

7.8.2 Atrazine determined in industrial effluent.

This part of the analysis was done by the department of General Chemistry at the SABS.

Conditions :

- **Instrument :** Hewlett Packard Series 5880A GC
- **Column used :** Megabore column 15m x 0.53 mm
- **Column temperature :** Isothermic T = 150°C
- **Detector :** Nitrogen phosphorus detector (NPD)

It should be noted that the above was all the information supplied for this analysis.

Chapter

8

Estrogen - mimics already found in South Africa.

An intensive literature survey was conducted to determine which of the estrogen and estrogen - mimicking substances were already found in South Africa. The results of this survey are given in table 8.1.

Telephone calls were made and faxes and e-mails were sent across the Country to different institutions, State departments, laboratories and universities in an effort to discover which substances were found in the environment, but of which no results were published yet. Although the response was slightly poor some insight was derived from it. The estrogen - mimicking substances found and reported by personal communications are listed in table 8.2.

Two maps were then compiled regarding the occurrence of estrogen and estrogen - mimics in the South African situation with regard to their occurrence in water (map 1) and their occurrence in animal tissue (map 2).

Table 8.1 : Summary of estrogen mimics found in the SA water environment (literature study).

Substance detected	Location	Sample	Levels found	Year	Source
alpha Endosulfan	Levubu River - Dongadziva, Kruger National Park	Water	1976 mg/l	74/01/21	van Dyk, L.P. 1978:77-80
alpha Endosulfan	Levubu River - Dongadziva, Kruger National Park	Water	6344 mg/l	74/03/17	van Dyk, L.P. 1978:77-80
alpha Endosulfan	Lataba River - Mahlangene, Kruger National Park	Water	104 mg/l	74/02/03	van Dyk, L.P. 1978:77-80
alpha Endosulfan	Crocodile River - Crocodile bridge, Kruger National Park	Water	97 mg/l	74/01/07	van Dyk, L.P. 1978:77-80
Arochlor 1254	Isipingo Estuary, Natal	Variety of fish species	96.5 µg/kg wet wt	1991	Grobler et al. 1996:572-575
Arochlor 1260	Loskop Dam - Olifants River, Eastern TM	Fish - E. intermedius	41.0 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
Atrazine	Vaal River (Villiers)	Water	0.13 µg/l	1987	Hassett et al. 1987:133-136
Atrazine	Wilge River (Frankfort)	Water	0.79 µg/l	1987	Hassett et al. 1987:133-136
Atrazine	Vaal River (Parys)	Water	ND	1987	Hassett et al. 1987:133-136
Atrazine	Vaal River (Balkfontein)	Water	0.38 µg/l	1987	Hassett et al. 1987:133-136
Atrazine	Laaispruit (Marquard)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Atrazine	Meulspruit (Ficksburg)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Atrazine	Liebenbergvlei River (Bethlehem)	Water	0.44 µg/l	1987	Hassett et al. 1987:133-136
Atrazine	Liebenbergsvlei (Reitz)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Atrazine	Renoster River (Viljoenskroon)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Atrazine	Vals River (Kroonstad)	Water	0.27 µg/l	1987	Hassett et al. 1987:133-136
Atrazine	Vet River (Hoopstad)	Water	0.24 µg/l	1987	Hassett et al. 1987:133-136
Atrazine	Sand River (Senekal)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Atrazine	Leeu River (Armenia dam)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Atrazine	Caledon River (Ladybrandt)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Atrazine	Caledon River (Welbedacht dam)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Atrazine	Not indicated	Rain water	Varies	1990	Coetzee & Vahrmeijer 1986:78
Atrazine	Balkfontein area, OVS	Surface water	82.3 µg/l	1986	Hassett et al. 1987:133-136
Atrazine	Zuikerbosh pump station, Vereeniging	Drinking water	< 0.05 to 4.59 µg/l	1986	Coetzee & Vahrmeijer 1986:87
Atrazine	Wenzel Dam in Harts River (Sweizer-Rencke)	Dam water	< 0.05 to 3.81 µg/l	1986	Coetzee & Vahrmeijer 1986:81-87
Atrazine	Bloemhof Dam in Vaal River (Bloemhof)	Dam water	< 0.05 to 6.18 µg/l	1986	Coetzee & Vahrmeijer 1986:81-87
Atrazine	Strydom Dam in Vals River (Kroonstad)	Dam water	< 0.05 to 11.48 µg/l	1986	Coetzee & Vahrmeijer 1986:81-87
Atrazine	Koppies Dam in Renoster River (Koppies)	Dam water	< 0.05 to 4.82 µg/l	1986	Coetzee & Vahrmeijer 1986:81-87
Atrazine	Loskop Dam in Olifants River (Middelburg)	Dam water	< 0.05 to 3.89 µg/l	1986	Coetzee & Vahrmeijer 1986:81-87
Atrazine	Vaal Dam in Vaal River (Deneysville)	Dam water	< 0.05 to 4.73 µg/l	1986	Coetzee & Vahrmeijer 1986:81-87
Atrazine	Not specified	Dam water	< 0.05 to 3.22 µg/l	1986	Coetzee & Vahrmeijer 1986:81-87
beta Endosulfan	Levubu River - Dongadziva, Kruger National Park	Water	5547 mg/l	74/03/17	van Dyk, L.P. 1978:77-80
beta Endosulfan	Lataba River - Mahlangene, Kruger National Park	Water	559 mg/l	74/02/03	van Dyk, L.P. 1978:77-80
beta Endosulfan	Crocodile River - Crocodile bridge, Kruger National Park	Water	1272 mg/l	74/12/11	van Dyk, L.P. 1978:77-80
BHC, p,p' DDE, p,p' DDT & Dieldrin	Transvaal	Guineafowls & Francolins	Varies	1978	Pick et al. 1981:1243-1251
BHC, p,p' DDE, p,p' DDT & Dieldrin	Transvaal	Various dead birds	Varies	1978	Pick et al. 1981:1243-1251
Chlorinated pesticides (excl. atrazine)	Vaal River (Villiers)	Water	0.27 µg/l	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Wilge River (Frankfort)	Water	0.18 µg/l	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Vaal River (Parys)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Vaal River (Balkfontein)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Laaispruit (Marquard)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Meulspruit (Ficksburg)	Water	Not indicated	1987	Hassett et al. 1987:133-136

Table 8.1 : Summary of estrogen mimics found in the SA water environment (literature study).

Substance detected	Location	Sample	Levels found	Year	Source
Chlorinated pesticides (excl. atrazine)	Liebenbergsvlei (Bedlêchem)	Water	0.11 µg/l	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Renoster River (Vrijensfontein)	Water	0.09 µg/l	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Vals River (Kroonstad)	Water	0.13 µg/l	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Vet River (Hoopsstad)	Water	0.12 µg/l	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Sand River (Senekal)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Lecu River (Armenia dam)	Water	0.12 µg/l	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Caledon River (Ladybrand)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Chlorinated pesticides (excl. atrazine)	Caledon River (Welbedacht dam)	Water	Not indicated	1987	Hassett et al. 1987:133-136
Chlorophenolic compounds	Isipingo Estuary, Natal	Water	Individual varieties	1991	Grobler et al. 1996:572-575
DDE & Dieldrin	Eastern Cape	Eggs of coastal birds	Varies	1984	de Kock & Randall 1984:193-201
DDE & PCBs	S.A. West coast	Blubber of Cape Fur Seal	3650 & 3520 µ/kg wet mass	1976	Gardner 1984:43-44
DDE & PCBs	Namibia coast	Blubber of Cape Fur Seal	1250 & 130 µ/kg wet mass	1976	Gardner 1984:43-44
DDE & PCBs	Natal coast	Blubber of porpoise	11400 & 16000 µ/kg wet mass	1979	Gardner 1984:43-44
DDE & PCBs	S.A. South Coast	Blubber of porpoise	870 & 0 µ/kg wet mass	1979	Gardner 1984:43-44
DDE, DDD & DDT	Wilderness Lakes Systems, Southern Cape Province	Varies	Varies	1985	de Kock 1985:198
DDE, DDD, DDT and PCBs	Gonubie River near East London	Varies	Varies	1985	de Kock 1985:198
DDE, DDD, DDT, PCBs & Dieldrin	Nahoon River near East London	Varies	Varies	1985	de Kock 1985:198
DDE, DDD, DDT, PCBs & Dieldrin	Wilderness Lakes System, Southern Cape Province	Varies	Varies	1985	de Kock 1985:198
DDE, DDD, DDT, PCBs & Dieldrin	North West province of S.A. : Baberspan/Klipdrift dam	Blubber of common dolphin	Varies	1985	de Kock 1985:198
DDE, DDD, DDT, PCBs & Dieldrin	Buffalo River near Laingsburg	Varies	Varies	1985	de Kock 1985:203
DDE, DDT, PCBs & Dieldrin	Matron Island	Feral domestic cats	Varies	1984	Gardner 1984:43-44
DDT	Letsaba River - Mahlangene, Kruger National Park	Water	500 mg/l	75/03/07	van Dyk L.P. 1978:77-80
DDT	Letsaba River - Mahlangene, Kruger National Park	Water	1000 mg/l	75/03/07	van Dyk L.P. 1978:77-80
DDT & Dieldrin	Transvaal	Penguin eggs	Mean 0.004 µ/g	1984	de Kock & Randall 1984:193-201
DDT & PCB levels	Wilderness Lakes Systems, Southern Cape Province	Heron & Raptors	Varies	1976	Peakall & Kemp 1976:139-141
Dieldrin	Wilderness Lakes Systems, Southern Cape Province	Varies	Varies	1987	de Kock & Boshoff 1987:413-416
Dieldrin	Letsaba River - Mahlangene, Kruger National Park	Water	1689 mg/l	74/02/03	van Dyk L.P. 1978:77-80
Dieldrin	Oltfants River - Oltfantskloof, Kruger National Park	Water	2852 mg/l	74/01/16	van Dyk L.P. 1978:77-80
Dieldrin	Wilderness Lakes System, Southern Cape Province	Brain tissue of birds	4-5 µg/g (average)	1987	de Kock & Boshoff 1987:413-416
Dieldrin	East Coast of SA, Durban area	Blubber of bottlenose dolphins	0-2.91 µg	1980-1987	Cockcroft et al. 1989:207-217
Dieldrin	Oltfants, Letsaba River & Lacort dam, Pretoria area	Cormorant	0.50 mg/kg fat	1978	Pick et al. 1981:1243-1251
Dieldrin	Oltfants River and dam & Letsaba River, Pretoria area	Fish : Barbel	0.87 mg/kg fat	1978	Pick et al. 1981:1243-1251
Dieldrin	Crocodile River, near Kaap Muiden, Pretoria	Fish : Mudfish	0.51 mg/kg fat	1978	Pick et al. 1981:1243-1251
Dieldrin	Durban area	Mussels & mullet samples	7.7 & 13.5-40 µ/kg wet mass	1986	Sibbald 1986:318-321
Dieldrin	Oltfants River and dam & Letsaba River, Pretoria area	Fish : Kuiper	0.78 mg/kg fat	1978	Pick et al. 1981:1243-1251
Dieldrin, pp'DDE & PCBs	Cathedral peak - Pietermaritzburg area, Natal	Other seals	Varies	1992	Mason & Rowe-Kowe 1992:29-31
Endosulfan	Elands River - Loskop Dam area	Water	40 - 4146 ng/dm	1973-1974	van Dyk L.P. et al. 1977:71-76
Endosulfan	Oltfants River - Loskop Dam area	Water	10 - 1147 ng/dm	1973-1974	van Dyk L.P. et al. 1977:71-76
Hepachlor	Loskop Dam - Oltfants River, Eastern Tv	Fish : O. mossambicus	1.0 mg/kg muscle fat	Des-90	Grobler et al. 1996:572-575
Hepachlor	Isipingo Estuary, Natal	Varies	less than 0.170 µg/kg wet wt	1991	Grobler et al. 1996:572-575
Lindane	Isipingo Estuary, Natal	Variety of fish species	0.781 µg/kg wet wt	1991	Grobler et al. 1996:572-575

Table 8.1 : Summary of estrogen mimics found in the SA water environment (literature study).

Substance detected	Location	Sample	Levels found	Year	Source
o,p' DDD	Loskop Dam - Lombards Bay, Eastern Tvl	Fish : E. intermedius	1.8 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
o,p' DDD	Loskop Dam - Lombards Bay, Eastern Tvl	Fish : E. intermedius	1.0 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
o,p' DDT	Loskop Dam - Phalaborwa Barrage, Eastern Tvl	Fish : E. intermedius	22.0 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
o,p' DDT	Isipingo Estuary, Natal	Variety of fish species	less than 0.11 µg/kg wet wt	1991	Grobler et al. 1996:572-575
p,p' DDD	Loskop Dam - Lombards Bay, Eastern Tvl	Fish : C. gariepinus	2.5 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDD	Loskop Dam - Phalaborwa barrage, Eastern Tvl	Fish : C. gariepinus	3.7 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDD	Loskop Dam - Olifants River, Eastern Tvl	Fish : E. intermedius	1.4 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDD	Isipingo Estuary, Natal	Variety of fish species	2.45 µg/kg wet wt	1991	Grobler et al. 1996:572-575
p,p' DDE	Wilderness Lakes Systems, Southern Cape Providence	Bird samples	Varies	1987	de Kock & Boshoff 1987:413-416
p,p' DDE	Olifants, Letaba River & Lacot dam, Pretoria area	Commonat	11.98 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDE	Olifants River and dam & Letaba River, Pretoria area	Fish : Barbel	14.67 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDE	Olifants River and dam & Letaba River, Pretoria area	Fish : Kurper	9.61 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDE	Olifants River, Pretoria area	Fish : Yellowfish	9.18 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDE	Crocodile River, near Kaap Muiden, Pretoria	Fish : Mudfish	3.79 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDE	Isipingo Estuary, Natal	Variety of fish species	15.1 µg/kg wet wt	1991	Grobler et al. 1996:572-575
p,p' DDT		Fish : O. mossambicus	8.4 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT		Fish : O. mossambicus	3.1 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT	Loskop Dam - Lombards Bay, Eastern Tvl	Fish : C. gariepinus	22.2 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT		Fish : E. intermedius	48.6 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT		Fish : O. mossambicus	5.3 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT		Fish : E. intermedius	92.3 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT	Loskop Dam - Olifants River, Eastern Tvl	Fish : C. gariepinus	26.0 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT	Loskop Dam - Olifants River, Eastern Tvl	Fish : E. intermedius	2.9 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT	Loskop Dam - Lombards Bay, Eastern Tvl	Fish : C. gariepinus	1.7 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT		Fish : E. intermedius	4.9 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT	Loskop Dam - Phalaborwa barrage, Eastern Tvl	Fish : C. gariepinus	2.2 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT		Fish : E. intermedius	76.0 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT	Olifants, Letaba River & Lacot dam, Pretoria area	Cormorant	0.26 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDT	Olifants River and dam & Letaba River, Pretoria area	Fish : Barbel	0.78 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDT	Olifants River and dam & Letaba River, Pretoria area	Fish : Kurper	2.15 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDT	Crocodile River, near Kaap Muiden, Pretoria	Fish : Mudfish	0.43 mg/kg fat	1978	Pick et al. 1981:1243-1251
p,p' DDT	Croc. River : Braam Raubenheimer Dam, Nelspruit area	Muscle tissue of fish	3.9-96.7 µg/kg	1994	Roux et al. 1994:333-340
p,p' DDT	Croc. River : Matatfin Weir, Nelspruit area	Muscle tissue of fish	8.9-56.8 µg/kg	1994	Roux et al. 1994:333-340
p,p' DDT	Croc. River : Lions Club, Nelspruit area	Muscle tissue of fish	27.3-144.0 µg/kg	1994	Roux et al. 1994:333-340
p,p' DDT	Croc. River : Ten Bosch Weir, Kamatipoort area	Muscle tissue of fish	0.1-181.0 µg/kg	1994	Roux et al. 1994:333-340
p,p' DDT	Isipingo Estuary, Natal	Variety of fish species	less than 0.23 µg/kg wet wt	1991	Grobler et al. 1996:572-575
p,p' DDT	Vaal River (Villiers)	Water	0.18 µg/l	1987	Hassett et al. 1987:133-136
p,p' DDT	Vaale River (Frankfort)	Water	0.17 µg/l	1987	Hassett et al. 1987:133-136
p,p' DDT	Vaal River (Parys)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Vaal River (Balkfontein)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Laispruit (Marquard)	Water	Not indicated	1987	Hassett et al. 1987:133-136

Table 8.1 : Summary of estrogen mimics found in the SA water environment (literature study).

<i>Substance detected</i>	<i>Location</i>	<i>Sample</i>	<i>Levels found</i>	<i>Year</i>	<i>Source</i>
p,p' DDT	Meulspruit (Ficksburg)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Liebenbergsvlei River (Reitz)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Liebenbergsvlei (Bethlehem)	Water	0.07 µg/l	1987	Hassett et al. 1987:133-136
p,p' DDT	Renoster River (Viljoenskroon)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Vals River (Kroonstad)	Water	0.1 µg/l	1987	Hassett et al. 1987:133-136
p,p' DDT	Vet River (Hoopstad)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Sand River (Senekal)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Leeu River (Armenia dam)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Caledon River (Ladybrandt)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDT	Caledon River (Welbedacht dam)	Water	Not indicated	1987	Hassett et al. 1987:133-136
p,p' DDD		Fish : E. intermedius	1.7 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDD		Fish : O. mossambicus	1.9 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDD		Fish : E. intermedius	13.2 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
p,p' DDT	Loskop Dam - Phalaborwa barrage, Eastern TM	Fish : C. gariepinus	10.2 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
PCB's	Wilderness Lakes Systems, Southern Cape Providence	Brain tissue of birds	0.06 µg (average)	1987	de Kock & Boshoff 1987:413-416
PCB's	Eastern Cape	Eggs of coastal birds	Varies	1984	de Kock & Randall 1984:193-201
PCB's	East Coast of SA, Durban area	Blubber of Bottlenose dolphins	0.09-67.18 µg	1980-1987	Cockcroft et al. 1989:207-217
PCB's & DDE	Kromme River near St. Francis Bay	Variety of fish species	Varies	1985	de Kock 1985:198
PCB's, DDE, DDD, DDT	Gamtoos River near Humansdorp	Variety of fish species	Varies	1985	de Kock 1985:198
PCB's, DDE, DDD, DDT	Swartkops River near Uitenhage	Variety of fish species	Varies	1985	de Kock 1985:198
PCB's, DDE, DDD, DDT	Great Fish River near Port Alfred	Variety of fish species	Varies	1985	de Kock 1985:198
PCB's, DDE, DDD, DDT	Bushmans River near Alexandria	Variety of fish species	Varies	1985	de Kock 1985:198
PCB's, DDE, DDD, DDT & chlordane	Sundays River, Graaf Reinet area	Variety of fish species	Varies	1985	de Kock 1985:198
PCB's, t-DDT, Dieldrin & chlordane	South - East Indian Ocean	Blubber of dolphins	Varies	1989	de Kock & Lord 1989:133-138
t-DDT	Loskop Dam - Olifants River, Eastern TM	Fish : C. gariepinus	26.0 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT		Fish : O. mossambicus	8.4 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT	Loskop Dam - Lombards Bay, Eastern TM	Fish : C. gariepinus	26.40 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT		Fish : E. intermedius	56.20 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT		Fish : O. mossambicus	9.40 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT		Fish : E. intermedius	202 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT		Fish : O. mossambicus	3.10 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT	Loskop Dam - Phalaborwa barrage, Eastern TM	Fish : C. gariepinus	16.10 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT		Fish : E. intermedius	205 mg/kg muscle fat	Des-90	Grobler, D.F. 1994:187-194
t-DDT	Wilderness Lakes Systems, Southern Cape Providence	African Fish Eagle egg	0.21 µg (wet weight)	1985	de Kock & Lord 1989:133-138
t-DDT	East Coast of SA, Durban area	Blubber of Bottlenose dolphins	0-85.66 µg	1980-1987	Cockcroft et al. 1989:207-217
Total BHC	Olifants, Letaba River & Lacot dam, Pretoria area	Cormorant	0.25 mg/kg fat	1978	Pick et al. 1981:1243-1251
Total BHC	Olifants River and dam & Letaba River, Pretoria area	Fish : Barbel	0.40 mg/kg fat	1978	Pick et al. 1981:1243-1251
Total BHC	Olifants River and dam & Letaba River, Pretoria area	Fish : Kurper	0.23 mg/kg fat	1978	Pick et al. 1981:1243-1251
Total BHC	Olifants River, Pretoria area	Fish : Yellowfish	0.26mg/kg fat	1978	Pick et al. 1981:1243-1251
Total BHC	Crocodile River, near Kaap Muiden, Pretoria	Fish : Mudfish	0.19 mg/kg fat	1978	Pick et al. 1981:1243-1251
Total DDT	Isipingo Estuary, Natal	Variety of fish species	20 µg/kg wet wt	1991	Grobler et al. 1996:572-575
Total DDT	Wilderness Lakes Systems, Southern Cape Providence	Fish tissue	110 µg/kg wet wt	1987	de Kock & Boshoff 1987:413-416

Table 8.1 : Summary of estrogen mimics found in the SA water environment (literature study).

<i>Substance detected</i>	<i>Location</i>	<i>Sample</i>	<i>Levels found</i>	<i>Year</i>	<i>Source</i>
Total DDT	Pongolo River	Fish : Tigerfish	91.8 µg/kg wet wt	1990	Bouwman et al. 1990:1-12
Total DDT		Mozambique tilapia	33.4 µg/kg wet wt	1990	Bouwman et al. 1990:1-12
Total DDT		Butter catfish	26.4 µg/kg wet wt	1990	Bouwman et al. 1990:1-12
Total DDT	Olifants River, Groblersdal area	Catfish	22.8 µg/kg wet wt	1994	Grobler 1994:187-194
Total DDT		Mozambique tilapia	7.0 µg/kg wet wt	1994	Grobler 1994:187-194
Total DDT		Butter catfish	154.6 µ/kg wet wt	1994	Grobler 1994:187-194
Total DDT	Isipingo Estuary	Fish tissue	20 µg/kg wet wt	1991	Grobler et al. 1996:572-575
Total DDT	Kosi Bay	Fish : Mugil cephalus	514 µg/kg wet wt	1974	Butler et al. 1983:127-135
Total DDT		Fish : Acnathopagrus berda	513 µg/kg wet wt	1974	Butler et al. 1983:127-135
Total DDT	Wilderness Lakes Systems, Southern Cape Providence	Fish : Lychia amia	100 µg/kg wet wt	1983	de Kock & Boshoff 1987:413-416
Total DDT		Fish : O. mossambicus	1-30 µg/kg wet wt	1983	de Kock & Boshoff 1987:413-416
Total DDT	Olifants River, Groblersdal area	Fish : O. mossambicus	7 µg/kg wet wt	1994	Grobler 1994:187-194
Total DDT	Isipingo Estuary	Fish : O. mossambicus	4.9 µg/kg wet wt	1996	Grobler et al. 1996:572-575
Total DDT residues	Wilderness Lakes Systems, Southern Cape Providence	Fish : Mozambique tilapia	0.009microg/l	1987	de Kock & Boshoff 1987:413-416
Total DDT residues		Fish : Southern mullet	0.034 µ/l	1987	de Kock & Boshoff 1987:413-416
Total DDT residues		Fish : Kob	0.009 µ/l	1987	de Kock & Boshoff 1987:413-416
Total DDT residues		Fish : Groovy mullet	0.04 µ/l	1987	de Kock & Boshoff 1987:413-416
Total DDT residues		Fish : Cape silverside	0.02 µ/l	1987	de Kock & Boshoff 1987:413-416
Total DDT residues		Fish : Freshwater mullet	0.63 µ/l	1987	de Kock & Boshoff 1987:413-416
Total DDT residues		Fish : Cape shumpnose	0.06 µ/l	1987	de Kock & Boshoff 1987:413-416
Total DDT residues		Fish : Flathead mullet	0.11 µ/l	1987	de Kock & Boshoff 1987:413-416
Total Endosulfan	Olifants, Letaba River & Lacot dam, Pretoria area	Cormorant	0.68 mg/kg fat	1978	Pick et al. 1981:1243-1251
Total Endosulfan	Olifants River and dam & Letaba River, Pretoria area	Fish : Barbel	1.09 mg/kg fat	1978	Pick et al. 1981:1243-1251
Total Endosulfan	Olifants River and dam & Letaba River, Pretoria area	Fish : Kurper	0.17 mg/kg fat	1978	Pick et al. 1981:1243-1251
Total Endosulfan	Olifants River, Pretoria area	Fish : Yellowfish	5.88 mg/kg fat	1978	Pick et al. 1981:1243-1251

Table 8.2 : Summary of estrogen and estrogen - mimics found in the South African water environment (personal communications).

Substance detected	Location	Sample	Levels found	Year	Source
4-nonylphenol	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
4-nonylphenol-di-ethoxylates	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
4-nonylphenoxycarboxylic acid	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
4-octylphenol	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
2,4 D	Wilge River/Vaaldam/Vaal River	Tested for (not found at the limit of detection : 2 µl/l)	Not indicated	18/11/93	SABS (1993)
2,4 D	Wilge River/Vaaldam outlet	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	26/11/92	SABS (1993)
Aldrin	Durban	Water	Test for: Some found 36-50 ppb		Mrs. S. Jackson (1996)
Aldrin		? Used in recent mosquito control	Not indicated		Dr. Spark (1996)
Aldrin	Johannesburg area	Haven't found	Not indicated		Cydna Laboratories (1996)
Aldrin		Test for	Below 20 ng/l		Ungeni Water (1996)
Aldrin	Wilge River/Vaaldam/Vaal River	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	18/11/93	SABS (1993)
Aldrin	Wilge River/Vaaldam outlet	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	26/11/92	SABS (1993)
Aldrin	Voelvlei waterplant, Cape Town	Raw water	2.7 ng/l	93/06/15	Cape Town Municipality (1996)
Aldrin	No. 7, Liesbeeck River, Cape Town	Water	3.2 ng/l	95/01/16	Cape Town Municipality (1996)
Aldrin	No. 9, Liesbeeck River, Cape Town	Water	4.0 ng/l	95/01/16	Cape Town Municipality (1996)
Aldrin	Lake, Liesbeeck River, Cape Town	Water	12.4 ng/l	95/01/16	Cape Town Municipality (1996)
Aldrin	Greenpoint Outfall	Sewage water	Varies	1984-1996	Cape Town Municipality (1996)
alpha & beta Endosulfan	Wilge River/Vaaldam/Vaal River	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	18/11/93	SABS (1993)
alpha & beta Endosulfan	Wilge River/Vaaldam outlet	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	26/11/92	SABS (1993)
alpha & gamma Chlordane	Greenpoint Outfall	Sewage water	Varies	1984-1996	Cape Town Municipality (1996)
alpha Chlordane	Wemmershoek waterplant, Cape Town	Raw water	0.4 ng/l	93/06/22	Cape Town Municipality (1996)
alpha Chlordane	Steenbras waterplant, Cape Town	Raw water	2.4 ng/l	93/06/23	Cape Town Municipality (1996)
alpha Chlordane	Constansia Neck waterplant, Cape Town	Raw water	0.9 ng/l	93/06/23	Cape Town Municipality (1996)
alpha Chlordane	Blackheath waterplant, Cape Town	Raw water	0.8 ng/l	93/06/15	Cape Town Municipality (1996)
alpha Chlordane	Kloof Nek waterplant, Cape Town	Raw water	0.6 ng/l	93/06/23	Cape Town Municipality (1996)
Alkylphenolic ethoxylates (APE's)	Johannesburg area	Haven't looked for them	Not indicated		Cydna Laboratories (1996)
Arochlor 1254		Uses in capacitors	Not indicated		Polifin (1996)
Atrazine		Surface & ground waters	Vary from sub-ppb to mg/l		Dr. P. Kempster (1996)
Atrazine	Vaalharts Irrigation scheme	Water	Varies	1987-1989	Mrs H. Badenhorst (1996)
Atrazine	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
Atrazine	Wilge River/Vaaldam/Vaal River	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	18/11/93	SABS (1993)
Atrazine	Wilge River	Tested for and found	2 µl/l	26/11/92	SABS (1993)
Atrazine	Orange River (Klipdriëdam)	Water	Not indicated	1990	Mrs H. Badenhorst (1997)
Atrazine	Mazelspoortstuwal	Water	Not indicated		
Atrazine	Kalkfontein dam	Water	all less than 0.03 µl/l		
Atrazine	Rusfontein dam	Water	Not indicated		
Atrazine	Modderiver at Jakobsdal	Water	Not indicated		
Atrazine	Rietrivier before and after	Water	Not indicated		
Atrazine	Vaalriver (Vaaldam)	Water	0.76 µl/l	1987	Mrs H. Badenhorst (1997)
Atrazine	Upper barrage	Water	none		
Atrazine	Lower barrage	Water	none		
Atrazine	Parys	Water	none		

Table 8.2 : Summary of estrogen and estrogen - mimics found in the South African water environment (personal communications).

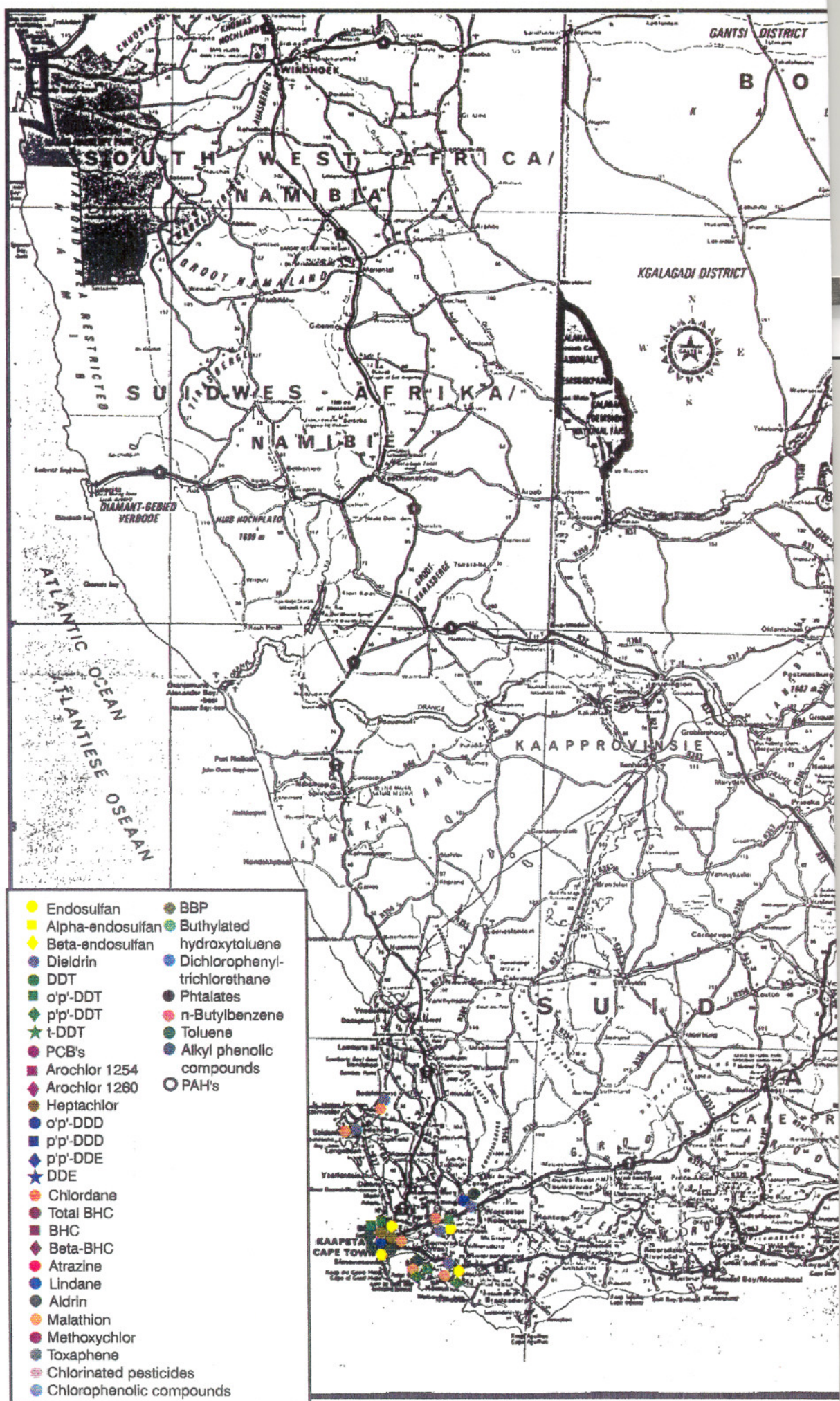
Substance detected	Location	Sample	Levels found	Year	Source
Atrazine	Orkney	Water	none		
Atrazine	Klipplaatdrift	Water	none		
Atrazine	Bloemhofdam	Water	0.4 µl/l		
Atrazine	Vaalhartswier	Water	0.8 µg/l		
Atrazine	Spitskopdam	Water	none		
Atrazine	Riverton	Water	none		
Atrazine	Douglas	Water	none		
Atrazine	Rodeplaatdam (Pretoria)	Water	Unknown	Unknown	Mrs H. Badenhorst (1997)
BBP	Crocodile River	Water	Not indicated	1984	Cape Town Municipality (1997)
Bisphenol A	Johannesburg area	Found in water	Not indicated		Cytha Laboratories (1996)
Butylated hydroxytoluene	Benoni / Durban area	Uses in compounding	Not indicated		Polifin (1996)
Butylated hydroxytoluene	Accidental pollution	Water	Not indicated		SABS (1996)
Chlordane	Wilde River/Vaaldam/Vaal River	Uses as an anti-oxidant	Not indicated		Polifin (1996)
Chlordane	Wilde River/Vaaldam outlet	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	18/11/93	SABS (1993)
Chlordane, dieldrin & lindane	Johannesburg area	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	26/11/92	SABS (1993)
Chlorophenols		Haven't found	Not indicated		Cytha Laboratories (1996)
Chlorophenols		due to chlorination of algae	Not indicated		
Chlorophenols	Johannesburg area	Sometimes in chlorinated waters	Often as much as several ppb		Dr. P. Kempster (1996)
DDE & dicofol	Johannesburg area	Absent	Not indicated		Cytha Laboratories (1996)
DDT	Accidental pollution	Not found	Not indicated		Cytha Laboratories (1996)
DDT		Water	Not indicated		SABS (1996)
DDT, heptachlor & malathion	Johannesburg area	? Used in recent mosquito control	Not indicated		Dr. Spark (1996)
Dieldrin	Durban	Not found	Not indicated		Cytha Laboratories (1996)
Dieldrin		Water	Test for: Some found 30-50 ppb		Mrs. S. Jackson (1996)
Dieldrin		? Used in recent mosquito control	Not indicated		Dr. Spark (1996)
Dieldrin	Sterk at Groothek	Water (pollution incident)	1714 ng/l	14/01/94	Ungeni Water (1996)
Dieldrin	Neulwane stream at weir	Water (pollution incident)	1270 ng/l	14/01/94	Ungeni Water (1996)
Dieldrin		Test for	Below 20 ng/l		Ungeni Water (1996)
Dieldrin	Wilde River/Vaaldam/Vaal River	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	18/11/93	SABS (1993)
Dieldrin	Wilde River/Vaaldam outlet	Tested for (not found at the limit of detection : 1 µl/l)	Not indicated	26/11/92	SABS (1993)
Dieldrin	Wemmershoek waterplant, Cape Town	Raw water	8.2 ng/l	92/10/06	Cape Town Municipality (1996)
Dieldrin	Voelvlei waterplant, Cape Town	Raw water	7.8 ng/l	92/10/06	Cape Town Municipality (1996)
Dieldrin	Steenbras waterplant, Cape Town	Raw water	19.4 ng/l	93/06/23	Cape Town Municipality (1996)
Dieldrin	Constansia Nek waterplant, Cape Town	Raw water	11.5 ng/l	93/06/23	Cape Town Municipality (1996)
Dieldrin	Kloof Nek waterplant, Cape Town	Raw water	10.2 ng/l	93/06/23	Cape Town Municipality (1996)
Dieldrin	Greenpoint Outfall	Raw water	Varies	1984-1996	Cape Town Municipality (1996)
Endosulfan	Durban	Sewage water	Test for: Some found 30-50 ppb		Mrs. S. Jackson (1996)
Endosulfan 1	Wemmershoek waterplant, Cape Town	Water	7.2 ng/l	92/10/06	Cape Town Municipality (1996)
Endosulfan 1	Lake, Liesbeek River, Cape Town	Raw water	21.2 ng/l	95/01/16	Cape Town Municipality (1996)
Endosulfan 1&2	Greenpoint Outfall	Water	Varies	1984-1996	Cape Town Municipality (1996)
Endosulfan 2	Steenbras waterplant, Cape Town	Sewage water	6.4 ng/l	93/06/23	Cape Town Municipality (1996)

Table 8.2 : Summary of estrogen and estrogen - mimics found in the South African water environment (personal communications).

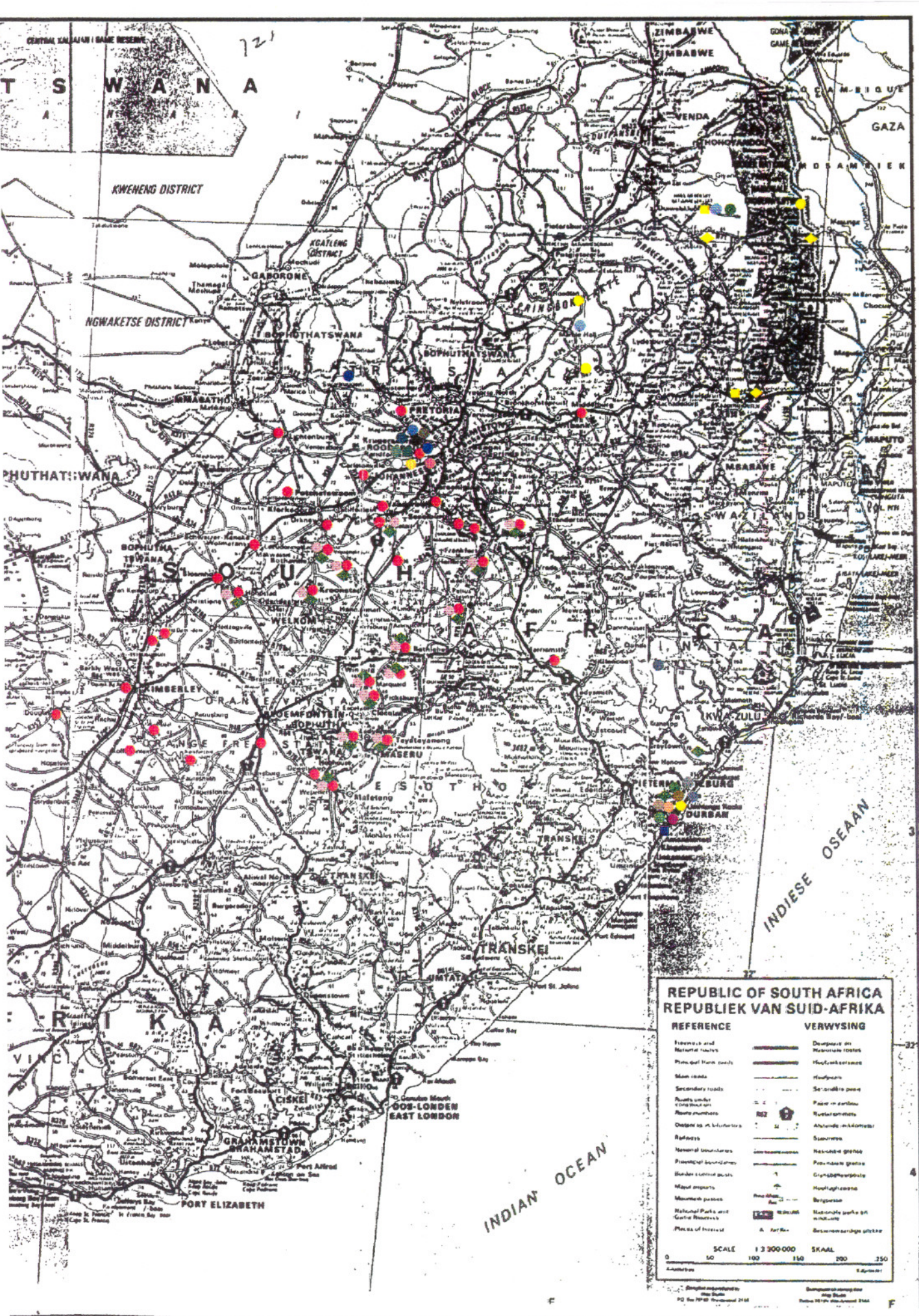
Substance detected	Location	Sample	Levels found	Year	Source
Endosulfan 2	No 7, Liesbeek River, Cape Town	Water	39.4 ng/l	95/01/16	Cape Town Municipality (1996)
Estrogen-like substances	Johannesburg area	Haven't looked for them	Not indicated	18/11/93	Cydnalaboratories (1996)
gamma BHC	Wilge River/Vaal River	Tested for (not found at the limit of detection 1 µl/l)	Not indicated	26/11/92	SABS (1993)
gamma BHC	Wilge River/Vaal River	Tested for (not found at the limit of detection 1 µl/l)	Not indicated	26/11/92	SABS (1993)
gamma BHC	Greenpoint Outfall	Sewage water	Varies	1984-1996	Cape Town Municipality (1996)
gamma BHC	Backhath waterplant, Cape Town	Raw water	0.5 ng/l	15/6/93	Cape Town Municipality (1996)
gamma Chlordane	No 7, Liesbeek River, Cape Town	Water	30.9 ng/l	95/01/16	Cape Town Municipality (1996)
Heptachlor	Greenpoint Outfall	Test for	Below 20 ng/l	1984-1996	Umgem Water (1996)
Heptachlor	Durban	Water	Test for. Some found 30-50 ppb	1984-1996	Cape Town Municipality (1996)
Heptachlor epoxide	No 7, Liesbeek River, Cape Town	Water	17.9 ng/l	95/01/16	Cape Town Municipality (1996)
Heptachlor epoxide	Lake, Liesbeek River, Cape Town	Water	182.9 ng/l	95/01/16	Cape Town Municipality (1996)
Heptachlor epoxide	Lindane	Test for	Below 20 ng/l	Umgem Water (1996)	Dr Spak (1996)
Lindane	Widespread	Test for	Below 20 ng/l	Umgem Water (1996)	Dr Spak (1996)
Lindane	Votlei waterplant, Cape Town	Raw water	2.2 ng/l	93/06/15	Cape Town Municipality (1996)
Lindane	Lake, Liesbeek River, Cape Town	Water	36.5 ng/l	95/01/16	Cape Town Municipality (1996)
Malathion	Durban	Water	Test for. Some found 30-50 ppb	1984-1996	Cape Town Municipality (1996)
Methoxychlor	Durban	Water	Test for. Some found 30-50 ppb	1984-1996	Cape Town Municipality (1996)
Methoxychlor	Johannesburg area	Soil & water samples	Not indicated	Dr Spak (1996)	Dr Spak (1996)
Methylphenol	Johannesburg area	Not found	Not indicated	Cydnalaboratories (1996)	Cydnalaboratories (1996)
n-Butylbenzene	Johannesburg area	Found in water	Not indicated	Cydnalaboratories (1996)	Cydnalaboratories (1996)
Nonylphenol	Greenpoint Outfall	Sewage water	Varies	1984-1996	Cape Town Municipality (1996)
o,p DDE	Greenpoint Outfall	Water	35.7 ng/l	95/01/16	Cape Town Municipality (1996)
o,p DDT	Greenpoint Outfall	Sewage water	Varies	1984-1996	Cape Town Municipality (1996)
o,p DDE	No 9, Liesbeek River, Cape Town	Water	35.7 ng/l	95/01/16	Cape Town Municipality (1996)
o,p DDT	Greenpoint Outfall	Sewage water	Varies	1984-1996	Cape Town Municipality (1996)
o,p DDE	Blackhath waterplant, Cape Town	Raw water	11.7 ng/l	92/10/06	Cape Town Municipality (1996)
o,p DDT	Wemmershoek waterplant, Cape Town	Raw water	22.0 ng/l	92/10/06	Cape Town Municipality (1996)
o,p DDT	No 7, Liesbeek River, Cape Town	Water	2.5 ng/l	95/01/16	Cape Town Municipality (1996)
o,p DDT & p,p DDT	Johannesburg area	Not found	Not indicated	Cydnalaboratories (1996)	Cydnalaboratories (1996)
p,p DDE	Stenbras waterplant, Cape Town	Raw water	32.8 ng/l	92/10/17	Cape Town Municipality (1996)
p,p DDE	Greenpoint Outfall	Raw water	Varies	1984-1996	Cape Town Municipality (1996)
p,p DDT	Greenpoint Outfall	Sewage water	Varies	1984-1996	Cape Town Municipality (1996)
p-tert-Butylbenzoic acid	Sasol (Karoochem)	Uses it	Not indicated	Cydnalaboratories (1996)	Karoochem (1996)
PAHs	Johannesburg area	Found in sewage effluent	Not indicated	Cydnalaboratories (1996)	Cydnalaboratories (1996)
PCBs	Widespread	Marine organisms in Durban harbor	Not indicated	Dr Spak (1996)	Dr Spak (1996)
PCBs	Found in dam in TVL and Natal	Found in dam in TVL and Natal	900 ppb - 32000 dpm	CSIR, Pretoria (1996)	CSIR, Pretoria (1996)
PCBs: dichlorophenyltrichloroethane	Goudkoppies, Northern Works, Jhb area	Not found in drinking water, only sewage effluent	Not indicated	Cydnalaboratories (1996)	Cydnalaboratories (1996)
PCBs: Aroclor 1254	Goudkoppies, Northern Works, Jhb area	Not found in drinking water, only sewage effluent	Not indicated	Cydnalaboratories (1996)	Cydnalaboratories (1996)
PDDFs & TIQ	Johannesburg area	Haven't looked for them	Not indicated	Cydnalaboratories (1996)	Cydnalaboratories (1996)
Phenols	Found as chips of pipes	Found as chips of pipes	Not indicated	Municipality of Pretoria (1996)	Municipality of Pretoria (1996)

Table 8.2 : Summary of estrogen and estrogen - mimics found in the South African water environment (personal communications).

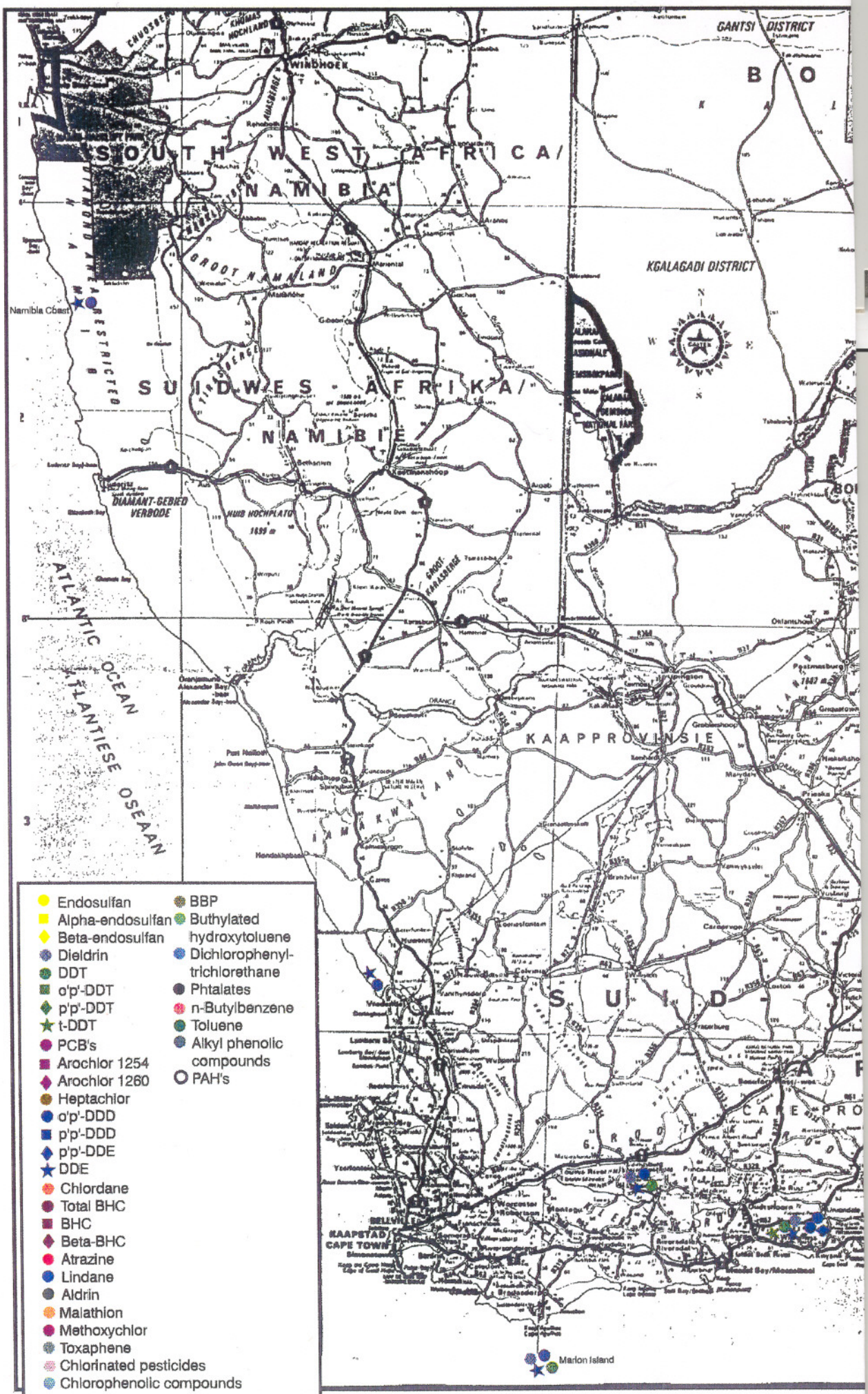
Substance detected	Location	Sample	Levels found	Year	Source
Phenoxyacetic acids & 2,4 D		Used to control grass verges in Natal	Not indicated		Dr. Spark (1996)
Phthalate: ethylhexylphthalate	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
Phthalates		Very common in water samples	Not indicated		Dr. P. Kempster (1996)
Phthalates	Universal		Not indicated		Dr. Spark (1996)
Phthalates	Benoni / Durban area	Uses in compounding	Not indicated		Polifin (1996)
Phthalates: diphenylphthalate	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
Phthalates: n-butyl phthalate	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
p,p' DDE	Wilge River/Vaaldam/Vaal River	Tested for (not found at the limit of detection : 1 µg/l)	Not indicated	18/11/93	SABS (1993)
p,p' DDE	Wilge River/Vaaldam outlet	Tested for (not found at the limit of detection : 1 µg/l)	Not indicated	26/11/92	SABS (1993)
p,p' DDE	Blackheath waterplant, Cape Town	Raw water	8.1 ng/l	92/10/06	Cape Town Municipality (1996)
p,p' DDT	Durban	Water	Test for. Some found 30-50 ppb		Mrs. S. Jackson (1996)
p,p' DDT	Umgeni Weir near Nagle dam, Pmb area	Water	98 ng/l	19/01/94	Umgeni Water (1996)
p,p' DDT	Duzi Eddy Hagan Drive	Water (pollution incident)	52 ng/l	19/01/94	Umgeni Water (1996)
p,p' DDT	Umgeni at Moortens Drift	Water (pollution incident)	85 ng/l	14/01/94	Umgeni Water (1996)
p,p' DDT	Sterk at Groothek	Water (pollution incident)	2256 ng/l	14/01/94	Umgeni Water (1996)
p,p' DDT	Nculwane stream at weir	Water (pollution incident)	1512 ng/l	14/01/94	Umgeni Water (1996)
p,p' DDT		Test for	Below 20 ng/l		Umgeni Water (1996)
p,p' DDT	Wilge River/Vaaldam/Vaal River	Tested for (not found at the limit of detection : 1 µg/l)	Not indicated	18/11/93	SABS (1993)
p,p' DDT	Wilge River/Vaaldam outlet	Tested for (not found at the limit of detection : 1 µg/l)	Not indicated	26/11/92	SABS (1993)
Toluene		Ground waters	ppb, near solid waste dumps		Dr. P. Kempster (1996)
Toluene	Johannesburg area	Found in water	Not indicated		Cydna Laboratories (1996)
Toxaphene	Durban	Water	Test for. Some found 30-50 ppb		Mrs. S. Jackson (1996)
Toxaphene		Used locally	Not indicated		Dr. Spark (1996)



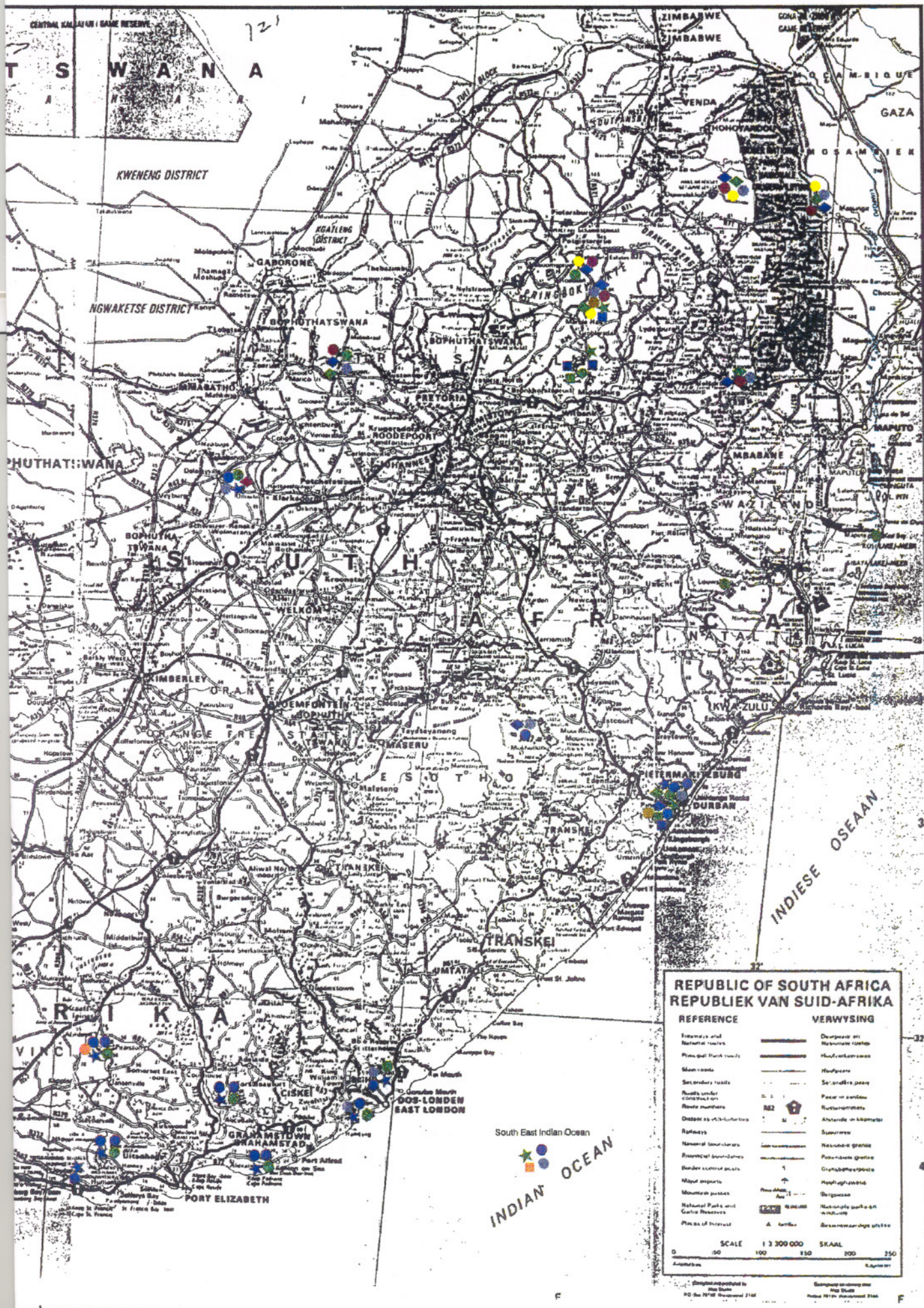
Map 1: Locations where estrogen and (drinking water, surface water, c



estrogen-mimicking substances were found in water sources
am water and sewage effluent).



Map 2: Locations where estrogen and endocrine-disrupting chemicals (EDCs) were found in fish tissue, dolphin tissue, bird tissue, and



strogen-mimicking substances were found in the animal kingdom (issue, mussels, otter scats and feral domestic cats).

Table 8.1 indicates that studies had been undertaken from as early as 1974, but without the knowledge that these substances were part of the estrogen - mimicking group.

Atrazine's widespread contamination is due to its wide usage as an agricultural agent in parts of the Country where crops are grown. Table 8.2 further amplifies this, indicating its widespread contamination in river and dam water also in these parts.

The occurrence of **DDT** in areas of the Kruger National Park and Natal can be explained due to the fact that DDT is used to prevent malaria in these areas.

Substances that are very lipophilic tend to accumulate more in the fats of animals (e.g. birds, dolphins, porpoises and fish) than in water, as is the case with **PCB's**, **DDT**, **dieldrin**, **heptachlor** and **endosulfan**. Thus, when a fish preys on insects, the DDT *bioaccumulates* in the fat content of the body and also when sediment is included in the diet of the fish. *Biomagnification* also plays a role in such that all the DDT that is in a bound state in the water (as discussed above) moves through the gills of the fish, making the contact with the lungs and thus the body, and fat content of the fish larger (Bouwman, 1997).

These two processes are dependant to a variety of factors such as pH, temperature, the type of fish and its eating habits, as well as the type of sediment involved.

However, their occurrence in water can possibly be explained due to the fact that substances like DDT adheres to dust particles and binds to particles in water like lipids, insects and humic acids (the substances that gives ground its brown colour) (Bouwman, 1997).

Chlorinated pesticide contamination correlates well with its usage in areas where agricultural activities are high.

Table 8.2 contained very relevant and useful information as it was directly linked to studies / investigations undertaken by various institutions and Water authorities regarding the tracing of these substances in the South African waters.

The information of table 8.1 and table 8.2 correlates to some extent.

From map 1 (the occurrence of estrogen and estrogen - mimics in water) it can clearly be seen that their localities lie spread throughout South Africa and being highly concentrated mainly in the PWV and surrounding areas. Atrazine once again seems to be the most prevalent contaminator. The reason being that agricultural activity in this area is high.

From map 2 (the occurrence of estrogen and estrogen - mimics in animal tissue) it is interesting to note that this occurrence is mainly spread and concentrated around the coastal areas and to a lesser extent in the North Eastern areas. This high concentration around the coastal areas are most likely due to the presence of more water surroundings and thus more water / sea life. Lipophilic substances such as **DDT**, **DDE** and **DDD** can be seen to contribute the most to the contamination of these areas.

Table 8.3 gives an indication of levels of estrogen and estrogen - mimicking substances that were found on an international level in water and animal tissues.

Table 8.3 : Estrogen and estrogen - mimicking substances found in water and animal tissues on an international level.

Estrogen / estrogen - mimic	Water source	Levels	Source	Location
Estrogen	Sewage effluent	1-10 ng/l	(ANON., 1993c:10)	England
Degradation products of detergents	Sewage effluent	Not indicated	(ANON., 1993c:10)	England
Plant estrogens	Sewage effluent	Not indicated	(ANON., 1993c:10)	England
Norethisterone	Sewage effluent	8-20 ng/l	(Aherne & Briggs, 1989:736)	South East England
	River water	< 2-17 ng/l	(Aherne & Briggs, 1989:736)	South East England
	Impounding reservoirs	< 2-10 ng/l	(Aherne & Briggs, 1989:736)	South East England
	Potable water	< 2-10 ng/l	(Aherne & Briggs, 1989:736)	South East England
Ethinyl estradiol	Sewage effluent	< 1-7 ng/l	(Aherne & Briggs, 1989:736)	South East England
	River water	2-15 ng/l	(Aherne & Briggs, 1989:736)	South East England
	Impounding reservoirs	1-3 ng/l	(Aherne & Briggs, 1989:736)	South East England
	Potable water	< 1-4 ng/l	(Aherne & Briggs, 1989:736)	South East England
Alkyl phenolic ethoxylates (APE)	Sewage discharges enters rivers	Up to 50 ng/l	(ANON., 1993a:24)	United Kingdom
	Treated drinking water		(ANON., 1993a:24)	United States
Ethinyl estradiol	Drinking water	0.06 ng/l	(Rathner & Sonneborn, 1979:48)	Netherlands
	River water	0.3 ng/l	(Rathner & Sonneborn, 1979:48)	Netherlands

Table 8.3 (continued) : Estrogen and estrogen -mimicking substances found in water and animal tissues on an international level.

Estrogen / estrogen - mimic	Water source	Levels	Source	Location
<i>Estrogen</i>	Raw sewage water	0.2-0.5 nmol/l	(Shore <i>et al.</i> , 1993:362)	Tel Aviv
<i>Estradiol & DES</i>	Drinking water	Varies	(Rurainski <i>et al.</i> , 1977:289)	Netherlands
<i>DDE & PCB's</i>	Blubber of Sea Lion	253000-34100 $\mu\text{g/kg}$ wet mass	(Gardner 1984:42)	California, USA
<i>Atrazine</i>	Water	Varies	(Coetzee & Vahrmeijer 1986:84)	Cedar River, IOWA USA
<i>Atrazine</i>	Water	Not indicated	(Coetzee & Vahrmeijer 1986:84)	River and lake, Italy
<i>Oestrone</i>	Domestic sewage effluent	Varies from site 1.4-76 ng/l	(ANON., 1996c:9)	United Kingdom
<i>17β-oestradiol</i>	Domestic sewage effluent	Varies from site 2.7-48 ng/l	(ANON., 1996c:9)	United Kingdom
<i>Etinyloestradiol</i>	Domestic sewage effluent	Varies from site 0.2-4.3 ng/l	(ANON., 1996c:9)	United Kingdom
<i>Nonylphenol (from Nonyl-phenol ethoxylates)</i>	Sewage treatment works, sea outfalls and industrial effluent plants	More than 1-2 $\mu\text{l/l}$ (presumed no effect level)	(ANON., 1996c:9)	Scotland
<i>15 Phtalate esters</i>	Sewage treatment works and sea outfalls	More than 1-2 $\mu\text{l/l}$ (presumed no effect level)	(ANON., 1996c:9)	Scotland
<i>4-Nonylphenol</i>	Sewage effluent	< 1 to 100 $\mu\text{l/l}$	(Lee & Peart, 1995:1976)	Canada
<i>60% of alkylphenolic poly-ethoxylates</i>	Aquatic environment	Not indicated	(White <i>et al.</i> , 1994:175)	United Kingdom

Chapter

9

Estrogen and estrogen - mimics detected in water samples.

9.1 Estrogen.

Unfortunately this part of the study was not successful due to poor recovery of samples, which made it impossible to detect ethinylestradiol in the suspected concentration range.

9.2 Alkyl phenolic compounds such as nonylphenol.

No nonylphenol was found in the drinking water of Johannesburg, Windhoek, Vereeniging or Pretoria.

Nonylphenol was found in the Vaalriver 0, 10 and 24 km and in the Sasol effluent.

Table 9.1 indicates the levels of nonylphenol found in the samples.

Table 9.1 : Nonylphenol found in water.

<i>Sample identification</i>	<i>Concentration nonylphenol (µg/l)</i>
Vaalriver - 0 km at the Barrage	2.0
Vaalriver - 10 km from the Barrage	4.0
Vaalriver - 24 km from the Barrage	5.0
Sasol effluent	11.0

It can be noted that the concentration of nonylphenol increased with the distance from the Barrage. This is due to the dilution of the effluent as it flows along the river.

The nonylphenol found in the Sasol effluent correlates well with the knowledge that was built up during the course of this investigation, namely that Sasol makes nonylphenol.

9.3 Organochlorine pesticides.

No organochlorine substances (DDT total, methoxychlor, endosulfan total, chlordane and dicofol) were found in the *Vaalriver samples, and Sasol (including Karbochem & Orchem)*.

No organochlorine content was found in the drinking water of Johannesburg, Windhoek, Vereeniging or Pretoria.

o'p' DDE, p'p' DDE and o'p' DDD were found in the *Polifin, Sanachem and AECl Industrial Chemicals (including Polifin)* sample.

Table 9.2 indicates the levels found of organochlorine pesticides.

Table 9.2 : Organochlorine pesticides found in water.

<i>Sample identification</i>	<i>Concentration o'p' DDE (µg/l)</i>	<i>Concentration p'p' DDE (µg/l)</i>	<i>Concentration o'p' DDD (µg/l)</i>
Polifin effluent	< 1.0	< 1.0	< 1.0
Sanachem effluent	< 1.0	< 1.0	< 1.0
AECl Industrial Chemicals (including Polifin)	< 1.0	< 1.0	< 1.0

A lindane (gamma BHC) concentration of 6.7 µg/l was also found in the *Sanachem* sample.

9.4 Organophosphate pesticides.

No malathion was found in any of the samples taken.

The lowest limit of detection for the drinking water tested was : < 10 µg/l.

9.5 Polychlorinated biphenyls (PCB's).

No PCB content was found in the drinking water of Johannesburg, Windhoek, Sasolburg or Vereeniging.

The lowest limit of detection for the drinking water tested was : < 5µg/l.

The PCB content that was tested for in the industrial effluent of SASTECH, in the Sasol area, and Sanachem from the Durban area, were decided to be the two PCB's : Arochlor 1254 and Arochlor 1260.

For the analysis of Arochlor 1254 six isomers were used, and for the analysis of Arochlor 1260 nine isomers were used for the quantitation.

When analysed for the PCB isomers the Sanachem sample indicated the presence of five isomers of Arochlor 1254 while the Sasol / SASTECH sample indicated the presence of only two isomers.

The Sanachem sample indicated the presence of eight isomers of Arochlor 1260, and the SASTECH / Sasol sample only three.

No PCB content was found in the Vaalriver samples, AECl Industrial Chemical (including Polifin) sample, or Polifin sample supplied.

Thus it can be concluded that Arochlor 1254 and Arochlor 1260 was found in the Sanachem and SASTECH / Sasol samples, being the highest in both cases in the Sanachem sample taken at Durban.

9.6 Triazine herbicides such as atrazine.

9.6.1 Atrazine found in dam water .

The levels of atrazine were as follows :

Table 9.3 indicates the levels of atrazine in dam water.

Table 9.3 : Atrazine found in water

Sample name	Concentration (µg/l)
Roodeplaat dam	0.416
Hartebeespoort dam	1.193
Vaalriver - 0 km from the Barrage	0.603
Vaalriver - 10 km from the Barrage	1.179
Vaalharts Irrigation Scheme	0.383
Boskop dam	< 0.25

It can clearly be seen that atrazine was found in all of the dams sampled at concentrations varying from as high as 1.193 µg/l (Hartebeespoort dam) to as to as low as < 0.25 µg/l (Boskop dam).

It should however be mentioned that some of the samples were taken shortly after rain, and can thus be lower than normal due to the diluting effect of the rain.

It should be noted that the atrazine concentrations is higher further from the Barrage indicating possible increased agricultural activity in this region.

9.6.2 Atrazine found in industrial effluent.

Apparent atrazine at a level between 2 and 10 µg/l was detected in the Sanachem and Sasol samples. Due to high residues of nitrogen containing compounds present in these samples, quantitative analysis was not possible.

9.6.3 Atrazine tested for in drinking water.

No atrazine content was found in the drinking water of Johannesburg, Windhoek, Vereeniging or Pretoria.

The lowest limit of detection was : < 2µg/l.

9.7 Other non estrogen -mimicking substances found in Industrial effluent.

Although not part of this study, it seems necessary to include the wide variety of substances found in the samples done by GC-MSD scan analysis - because it gives an indication of the level of pollution of water resources.

Table 9.4 indicates the non estrogen - mimics found in industrial effluent.

Table 9.4 : Non estrogen - mimics found in industrial effluent

<i>Sample identification</i>	<i>Compounds found</i>
Tap water (control)	Phthalic acid esters
AECI Industrial Chemicals (including Polifin)	Pentacosane, heptacosane and phthalic acid ester
Sanachem	1,2,4 - Trichlorobenzene, 7- bezofuranol, 2,3 dihydro-2,2-dimethylcarbamate, methylcarbamate, 2,6 -diethylbenzenamine, phthalic acid ester 1,2,4,5-Tetrachlorobenzene 1,3,5-Triazine-2,4,6-trione, 1,3,5-Trimethyl Alachlor (main compound)
Sasol (including Karbochem & Orchem)	1,2-Benzisothiazole, 2(3H)-Benzothiazolethione, phthalic acid ester 2-Benzothiazolesulfenamide, N-(1,1-dimethylethyl) main compound different alkylbenzene compounds
Vaalriver - 0 km form the Barrage	Decacosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane nonacosane, <i>phthalic acid ester - main compound</i> (also in control sample)
Vaalriver - 10 km form the Barrage	Phthalic acid esters - see control sample
Vaalriver - 24 km form the Barrage	Tricosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane, phthalic acid esters

Chapter

10

Summary and conclusions

From the *literature* it can clearly be seen that estrogen and estrogen - mimics found in the environment has a remarkable effect on the reproductive status of animals exposed. All of the observed effects like abnormal nesting behaviour, reproductive tracts that contain both male and female tissues in the *same* animal (hermaphrodism) the formation of vitellogenin in animals that don't normally produce this substance, and to a greater extent : sperm abnormalities such as : sperm malformations and decreased sperm motility, genital abnormalities such as chrytorchidism, smaller genital size in males, urethral abnormalities and abnormal hormone ratios with higher estrogen than testosterone levels in males, all indicate what the effect of exposure to these substances can be. Wildlife is sending a serious message to the human race that something placed in the environment has the potential of disrupting their ability to reproduce.

Also on the human side, increased instances of infertility - with the male now contributing more than ever towards this sensitive matter, increased instances of breast cancer and instances where pregnant mothers' consumption of fish (contaminated with an estrogen - mimic) affected their offspring should send a clear message to the human race that something in our surroundings and environment is working against our ability to reproduce.

Although there exists evidence in the literature that the situation isn't as serious as it seems, this evidence is in the minority.

From the investigation done with regard to the *literature survey applicable to the South African situation and the personal communications* made, there seems to be a large amount of substances that were already found in the water environment. These substances have been shown to have the

potential to disrupt the reproductive status. Many of these substances were found in the PWV area.

A lot of these substances like the PCB's : Arochlor 1254 and Arochlor 1260 and DDT are lipophilic and accumulate in the fat content of the body thus leading to concentration over a long period of time, even though the initial exposure was to minute quantities. This fact is often misunderstood and lethally discarded.

From the ***analyses done during the course of this investigation*** it can be seen that contamination of the environment does exist. Substances like nonylphenol, which has a proven effect on the lowering of the male reproductive status was found in the Vaalriver. Lindane, DDE and DDD content were found in industrial effluent, atrazine was found in dam and river water and the PCB's : Arochlor 1254 and 1260 were found in industrial effluent.

Most of the substances that were responsible for the contamination were of agricultural and industrial origin.

The drinking water samples analysed indicated that the present situation is that of a *safe* one. This can be due to the fact that the current procedures taken to remove substances for safe public consumption do remove these substances efficiently. The other side of the matter could be that the substances are present, but due to dilution are *under* the detection limit and can thus not be detected.

Although nonylphenol was not detected in the drinking water, there does exist very recent evidence recorded in two of the leading newspapers the *Beeld* (Korrespondent, 1997:25) and the *Rapport* (Erlank, 1997:5) that nonylphenol was found in drinking water. The reason for this discrepancy

could be that different locations were sampled at other time intervals than that of which this investigation was undertaken.

To sum up the present situation, one can conclude that the South African environment is definitely contaminated, and that some effects can already be seen in animals. At present there is no evidence that humans are affected.

Taking the present situation and this investigation in consideration a lot more research can be undertaken to assess the overall situation as it is in South Africa. *This investigation is far from being final and conclusive of the South African water environment, and can be seen only as being part of the tip of the iceberg.*

Proposed studies such as *cause and effect and in vivo studies* can be undertaken in order to determine at what levels the estrogen and estrogen - mimics can give an undoubtable effect on the human reproductive status as determined by using animals. Also, during this investigation *dioxins* were not tested for due to the fact that these determinations are very expensive and are largely done overseas. Dioxins have been proved to have an effect on the male reproductive system, and should be taken in consideration for a future investigation. *A study similar to that undertaken in the UK* to determine what the extent and levels of estrogenic compounds in the sewage systems are, can also be proposed. Also one could combine this with animal studies by measuring the vitellogenin levels of fish species that are placed in cages in the sewage effluent, and thus determining the relevant potency of these substances. Furthermore, one could try and *develop new methods of detection* or by *improving on standing methods* e.g. by improving on the limit of detection.

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Glossary

- 2,4 D -dichlorophenoxy acetic acid (herbicide)
- APE's : Alkyl phenolic ethoxylates
- Arochlor 1254 : a PCB mixture of which 54% is chlorinated.
- Arochlor 1260 : a PCB mixture of which 60% is chlorinated.
- Bisphenol A : a fungicide manufactured from phenol and acetone. This substance has two methyl groups on the carbon atom that separates the two phenol rings that each contains a hydroxy group.
- Bisphenol B : a substance used in the manufacture of phenolic resins and which is prepared from phenol and ethyl methyl ketone. This substance has an ethyl and a methyl group on the carbon atom that separates the two phenolrings which each contains a hydroxy group.
- Chlordecone : also known as kepone or CD
- Chlorinated hydrocarbons : substances like DDT, DDE and PCB's.
- Cryptorchidism : undescended testes in males
- DBP : bis(4-chlorophenyl)-methanonone
- DDD : 1,1 dichloro-2,2-bis(p-chlorophenyl)ethane :TDE
- DDE : dichlorodiphenyldichloro dichloroethylene (insecticide)
- DDT : dichlorodiphenyl trichloroethane (insecticide)
- DEHP : bis(ethylhexyl)phtalate
- DES : diethylstilboestrol, an exogeneous estrogen.
- Dioxin : 2,3,7,8 tetrachloro-p-dioxin, TCDD. One of 75 possible dibenzo-p-dioxin congeners. It is the most potent of the polychlorinated and polybrominated di-benzo-p-dioxins, dibenzofurans and biphenyls (PCB's), and as such serves as the prototype congener for toxicity elicited by these classes of chemicals.
- DMBA : dimethylbenz(a)anthracene

- Endogenous estrogens : estrogens found naturally inside of the human body
- Endometrial hypoplasia : a condition where the lining of the uterus is very thin.
- EPA : 5,8,11,14,17 - Eicopentaenoic acid - an important polysaturated fatty acid of the marine foodchain that serves as a precursor for the prostaglandin-3-and thromboxane-3-families.
- Exogeneous estrogens : synthetic estrogens that are given to a human to use for either contraception, or the treatment of some cancers e.g. ethinylestradiol, mestranol, estradiol phenylpropionate, diethylstilboestrol dipphosphate (DES), estradiol valerate and conjugated forms of estrogen.
- F.D.A. : Food and Drug Administration
- Hypoplastic testes : a condition where the testicles shrink to a very small size.
- Hypospadias : a condition where the opening of the male urethra is situated on an abnormal place in the human body.
- Isoflavonoids : defensive chemicals produced by legumes against cancer.
- Mycotoxins : a substance that is produced by *Fusarium graminearum*, a fungus.
- N.I.H. : National Institute of Health
- Osteoporosis : a silent disease where bones become brittle due to decreased bone formation and an estrogen shortage. Mainly found in women.
- PAH's : Poly aromatic hydrocarbons - are large, aromatic compounds sometimes found in air, water or other natural environments as a result of the combustion of petroleum products. These are monitored to maintain a healthy environment. E.g. anthracene, benz(a)anthracene, dibenz(a,h)anthracene.
- PCB's : Poly chlorinated biphenyls - a generic name for a group of chlorinated aromatic compounds with biphenyl as the basic structural group.

- PCDD : poly chlorinated dibenzo-p-dioxins
- Phtalates : Chemicals that are widely used as plasticisers.
- Phytoestrogens : plant substances that have estrogenic properties.
- Plasticisers : substances used in the plastic industry e.g. BBP, bisphenol A, DEHA, p-tert-butylbenzoic acid and phtalates.
- Resorcylic acid lactones : reproductive hormones produced by the genus *Fusarium* and are commonly found as contaminants of improperly stored grain.
- RU 486 : Mifepristone, an estrogen antagonist.
- TCDD : 2,3,7,8 tetra chloro-dibenzo-dioxins ; dioxin
- TCDD-EQ : tetrachlorodibenzo-p-dioxin equivalents
- TCDF : 2,3,7,8-tetrachlorodiebenzofuran, furan
- TIQ : tetrahydro-isoquinolines
- Vitellogenesis : the production of vitellogenin, a precursor of yolk, found in all egg laying animals after an estrogen stimulus is sent to the liver.
- Xenobiotics : substances like o'p' DDT, methoxychlor and chlordecone (kepone).
- Xenoestrogens : foreign estrogens e.g. kepone, PCB's, mirex ect.
- Zearalenone : the product of the fungus *Fusarium* that commonly infests corn.

Appendix 1 Chemical names and classification of estrogen and estrogen-mimics

CHEMICAL NAMES OF ESTROGEN AND ESTROGEN-MIMICKING SUBSTANCES

1. (1 α ,2 β ,2 α ,3 β ,6 β ,6 α ,7 β ,7 α)-3,4,5,6,9,9-hexachloro-1,2,2a,3,6,6a,7,7a-octahydro-2,7,3,6-dimethanoaphth-[2,3-b]oxirene
2. 1-hydroxychlordene
3. 2,2'-dihydroxy-5'-dichlorophenylphenol
4. 2,4-Dichlorophenoxy acetic acid (2,4 D) : broadleaf herbicide used in North America
5. 3,9-dihydroxybenz(a)anthracene (combustion product)
6. 3-adihydroxybeza[a]-anthracene
7. 4-nitrotoluene (manufacture of dyes)
8. Adiol : 5-androstene-3 β -17 β -diol
9. Aldrin (HHDN) : (1 α ,4 α ,4 α ,5 α ,8 α ,8 α)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene OR 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene (pesticide)
10. Alkyl phenolic compounds :
 4-octylphenol
 4-nonylphenol
 4-nonylphenol-di-ethoxylate
 4-nonylphenoxycarboxylic acid
11. Alkyl Phenols : pentaphenol to nonyl phenol
12. Alliquotes of the extracts tested for total estrogen activity in Medicago Sativa (α - α plant)
13. Anthocyanidins
14. APE (alkylphenolic ethoxylates)
15. APEC
16. APEO's : alkylphenolic poly ethoxylates eg. nonylphenolpolyethoxylates (80% of APEO's) & octylphenol poly ethoxylates (20% of APEO's)
17. Arochlor 1254 & 1260
18. Aromatic Hydrocarbons
19. Atrazine: 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5 triazine 2,4-diamine
20. β -HCH (β -hexachlorocyclohexane - isomeric contaminant formed during manufacturing of the insecticide lindane) : (1 α ,2 β ,3 α ,4 β ,5 α ,6 β)-1,2,3,4,5,6-hexachloro-cyclohexane
21. BBP (plastiziser, especially in the production of vinyl floors): 1,2-benzenedicarboxylic acid, esters, butyl phenylmethyl-esters
22. Benzo[a]pyrene: 3,4 benzpyrene : (combustion byproduct of foods and cigarettes)
23. Benzophenone : (manufacture of insecticides and anti histamines; fixture in strong perfumes, soaps and shampoos)
24. BHA : (1,1-dimethylethyl)-4-methoxy-phenol
25. Bisphenol A : 4,4'-(1-methylethylidene)bispheanol
26. Boron
27. Broccoli
28. Butylated hydroxytoluene (BHT) : (Anti oxidant in food packaging, PVC, cellulose and certain elastomers, and insect repellents)
29. Caffeine : (found in drinks and pharmaceuticals)
30. Carboxylic acid
31. Chalcones
32. Chicken manure
33. Chlordane (pesticide): 4,7, methano-1-H-indene -1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-chlordane
34. Chlordecone (commercial name : kepone, CD) : decachloro-octahydro-1,3,4-metheno-2H-cyclobuta-pentalen-2-one
35. Chlorinated Hydrocarbons
36. Chlorinated organic compounds:
 Atrazine
 Chlordane
 DDT
 Endosulfan
 Kepone
 Methoxychlor
 Some PCB's

37. Chlorine compounds that bleach paper
38. Cholesterol : (excreted steroid, emulsifying agent)
39. Cimetidine (SFK) : N-cyano-N'-methyl-N''-[2-[(5-methyl-1H-imidazol-4-yl)methyl]thioethyl-guanidine OR N-cyano-N''-methyl-N''-[2-[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]guanidine
40. Clenbuterol (NAB) : 4 amino-3,5-dichloro- α -[(1,1-dimethylethyl-amino)methyl]-benzene-methanol OR 4-amino- α -[(tert-butylamino)methyl]-3,5-dichloro-benzyl alcohol
41. Clone ID5 : anti-idiotypic antibody
42. Corn oil and related poly unsaturated fats
43. Coumestrol (most potent plant isoflavonoid) : 3,9-dihydroxy-6H-Benzofuro[3,2-c][1]benzopyran-6-one OR 3',5'-dihydroxy-coumestane OR 2-(2,4 -dihydroxyphenyl)-6-hydroxy-3-benzofurancarboxylic acid- gamma-lactone
44. Crops grown in animal pastures
45. Cycloheksimied : [1s-[1 α , (s*), 3 α , 5 β]]-4-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6 piperidinedione
46. DBP (dispersant in insect repellents, and found in plastic plumbing pipes and foodwraps) : bis (4-chlorophenyl)-methanonone
47. DDE : 1,1'-(dichloroethenylidene)bis [4-chloro-benzene]
48. DDT : 1,1'-(2,2,2-trichloroethylidene)bis[4-chloro-benzene] OR dichlorodiphenyltrichloroethane
49. DEHA : Bis-(2-ethyl-hexyl)adipate : (manufacture of plastics)
50. DEHP : Bis (ethyl-hexyl)phtalate) (source : plasticizer)
51. DES : (E)- 4,4'-(1,2-diethyl-1,2-ethenediyl) bis-phenol
52. Di(2)ethylhexyl)phtlates (plasticider for PVC)
53. Dichlorofenol (found in fungicide and germicide products)
54. Dicofof : 4-chloro- α -(4-chlorophenyl)- α - (trichloromethyl)-benzene-methanol
55. Dieldrin (pesticide) : (1 α , 2 β , 2 α , 3 β , 6 β , 6 α , 7 β , 7 α)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a,-octahydro-2,7,3,6 dimethanophtl[2,3b]oxirene OR 1,2,3,4,10,10 hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo OR exo-1,4:5,8 dimethanophtalane.
56. Dimethylphenol (disinfectant /microbicide)
57. Dioxins (herbicide contaminant) : Dibenzo[b,e][1,4]dioxin 2,3,7,8,tetrachloro dioxine (bactericide) : 1,3 dioxan -4-ol 2,6 dimethyl-dioxan-acetate
58. Diphenylphtalate
59. DMBA : 7,12 di methyl benz (a) anthracene
60. EPA : (antihyperlipoproteinemie) 5,8,11,14,17 - Eicopentaenoic acid
61. Endosulfan (α -isomer) : (3 α , 5 α β , 6 α , 9 α , 9 α)-6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro 3-oxide,6,9-methano-2,4,3,benzo-dioxathiepin
62. Endosulfan (β -isomer) : (3 α , 5 α β , 9 β , 9 α)-3 oxide-6,7,8,9,10,10-hexachloro -1,5,5a,6,9,9a-hexahydro-6,9, methano-2,4,3-benzodioxathiepin
63. Endosulfan: 6,9-methano-2,4,3,-benzodioxathiepin,6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro endosulfan -3-oxide OR 6,7,8,9,10,10 -hexachloro-1,5,5a,6,9,9a hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide OR 1,4,5,6,7,7-hexachloro-5-norbornene-2,3-dimethanol cyclic sulfite
64. Enterodiol : 2,3-bis[(3-hydroxyphenyl)methyl]-1,4-butanediol
65. Enterolactone : trans, dihydro-3,4-bis[3-hydroxyphenyl)methyl]-2(3H)-furanone
66. Epidermal Growth Factor (human) : 3L-aspartic acid-4-L-serine-5L-glutamic acid-8L-leucine-10L-histidine-16L-histidine-17L-aspartic acid-22L-tyrosine-25L-alanine-28L-lysine-30L-alanine-35L-valine-38L-isoleusine-40L-glutamicacid-44L-tyrisine-48L-lysine- α -epidermal growth factor.
67. Equol (phytoestrogen) : (s)-3,4 di hydro-3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol
68. Estrone : steroidal estrogen 3-hydroxyestra 1,3,5(10) triene -17-one
69. Estradiol : 1,3,5(10)-estratriene,3,17- β -diol-estradiol OR (8 α ,9 β ,13 α ,14 β ,17 α)-estrs 1,3,5 (10)-trine-3,17-diol
70. Estrogen found in sewage water
71. Estrone :3-dihydroxy-estra 1,3,5 (10)-triene-17-one
72. Ethinyloestradiol : (17a)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol.
73. Flanonols : (3-hydroxyflavnes)
74. Flavones : 3,hydroxy-2-phenyl-4H-1-benzopyran-4-one

75. Flavonones
76. Fuel constituents : such as aromatic hydrocarbons
77. Ginseng
78. Heptachlor
79. IgG
80. Isoflavones (genistein, daidzein, formononetin)
81. Isoflavones : 2,3-dihydro-3-phenyl-4H-1-Benzopyran-4-one
82. Kepone: 1,3,4-metheno-2H-cyclobuta [cd]-pentalen-2-one-1,1,3,3a,4,5,5a,6-decachloro octahydro-kepone
83. Lindane : (1 α ,2 α ,3 β ,4 α ,5 α ,6 β)-1,2,3,4,5,6-hexachloro-cyclohexane (gamma HCH) (broad spectrum insecticide used in a variety of agricultural, medical and veterinary products. In the USA it is a active component of a shampoo (Kwell) used for the treatment of pediculosis as well as for limited use on food crops)
84. Malathion :[(dimethoxyphosphinothiol)thio]butanedioic acid, diethyl ester OR mercaptosuccinic acid diethyl ester, s-ester with o,o-dimethyl phosphorothioate
85. Manure of swine, goats, horses, cows
86. Methoxychlor (herbicide) :1,1'-(2,2,2-trichloroethylidene)-bis-[4-methoxy-benzene]
87. Methylphenol (found in herbicide products / phenolic resins)
88. Mifepristone (RU 486) : 11-[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-(11 β , 17 β)-estra-4,9,dien-3-one OR (11 β , 17 β)-11-[4-(bi methylamino)-phenyl]-17-hydroxy-17-(1-propyl)estra-4,9-dien-3-one
89. Milk from pregnant cows
90. Milk
91. Mirex: 1,1a,2,2,3,3a,4,5,5a,5b,6-dodecachloroethahydro-1,3,4,-methano-1H-cyclobuta[cd]pentalene
92. Musk ketone (scent)
93. Musk xylene (scent)
94. Mycotoxins
95. n-Butylbenzene : (petrochemical origin)
96. Nonylphenol
97. Norethisterone :19-norpreg-4-en-20-yn-3-one,17-hydroxy(17 α)-norethiserone OR 17, hydroxy (17 α)-19-nor-pren-4-en-20-yn-3-one
98. o' Phenylfenol
99. o'p' DDD : 2 (o-chlorophenyl)-2-(p-chlorophenyl)-1,1-dichloroethylene
- 100.o'p'DDE : 2 (o-chlorophenyl)-2-(p-chlorophenyl)1,1,di chloroethylene
- 101.Octyl phenol
- 102.o'p'-DDT : 1-chloro-2-(2,2,2-trichloro-1-(4-chlorophenyl)ethylbenzene OR 2(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1, trichloroethane
- 103.Organochlorines
- 104.p'p' DDE : 2,2 bis-(p-chloro phenyl)-1,1-dichlorororthylene
- 105.p-Cresol (4-methylphenol) : to produce anti oxidant, UV stabilizer
- 106.p-Hydroxy benzoic acid (cosmetic, food and pharmaceutical preservative)
- 107.p'p'DDT :1,1'-(2,2,2-trichloroethylidene)bis[4-chloro-benzene] OR 2,2-bis-(p-chlorophenyl)-1,1,1 trichloroethane
- 108.p-tert-Butylbenzoic acid (plastic industry; corrosion inhibitor in polystyrene manufacture in dyeing)
- 109.Toluene (industrial solvent)
110. PAH (polycyclic aromatic hydrocarbons) : N-(4-aminobenzoyl)-glycine (aa)
- 111.Paper Mill wastes
- 112.PCB's (organochlorine compounds eg. dichlorodiphenyltrichloorthane and products of combustion eg. petrol engine) Eg. 2'4'6'trichlor-4-bi-phenylol and 2'3'4'5'tetrachloro-4-biphenylol
- 113.PCDD : Poly chlorinated dibenzo-p-dioxins
- 114.PDDF's : dibenzofurans
- 115.Pentachlorofenol
- 116.Petroleum by-products

CLASSIFICATION OF ESTROGEN AND ESTROGEN MIMICKING SUBSTANCES

• HERBICIDES

1. (2,4 Dichlorophenoxy) acetic acid (2,4 D) : broadleaf herbicide used in North America
2. Atrazine: 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5 triazine 2,4-diamine
3. Dioxins (herbicide contaminant) : Dibenzo[b,e][1,4]dioxin 2,3,7,8,tetrachloro dioxine
4. Methoxychlor (herbicide) : 1,1'-(2,2,2-trichlororhylidene)-bis[4-methoxy-benzene]
5. Methylphenol (found in herbicide products / phenolic resins)

• PESTICIDES /INSECTICIDES

1. Chlordecone (commercial name : kepone , CD) : decachloro-octahydro-1,3,4-metheno-2H-cyclobuta-pentalen-2-one.
2. DDT : 1,1'-(2,2,2-trichloroethylidene)bis[4-chloro-benzene] OR dichlorodiphenyltrichloroethane
3. Endosulfan : 6,9-methano-2,4,3,-benzodioxathiepin,6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro endosulfan -3-oxide OR 6,7,8,9,10,10 -hexachloro-1,5,5a,6,9,9a hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide OR 1,4,5,6,7,7-hexachloro-5-norbornene-2,3-dimethanol cyclic sulfite.
4. Heptachlor (insecticide for control of cotton boll weevil : 1H-1,4,5,6,7,8,8-Heptachloro-3a,4-7,7a-tetrahydro-4,7-methanoindene
5. Kepone: 1,3,4-metheno-2H-cyclobuta [cd]-pentalen-2-one-1,1,3,3a,4,5,5a,6-decachloro octahydro-kepone
6. Malathion (insecticide) : [(dimethoxyphosphinothiol)thio]butanedioic acid, diethyl ester OR mercaptosuccinic acid diethyl ester, s-ester with o,o-dimethyl phosphorothioate.
7. Mirex : (insecticide), common fire retardant for plastics, rubber, paint, paper and electrical goods) 1,1a,2,2,3,3a,4,5,5a,5b,6-dodecachloroethahydro-1,3,4,-methano-1H-cyclobuta[cd]pentalene.
8. o'p'-DDT : 1-chloro-2-(2,2,2-trichloro-1-(4-chlorophenyl)ethylbenzene OR 2(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1,1-trichloroethane
9. p'p' DDT : 1,1'-(2,2,2-trichloroethylidene)bis[4-chloro-benzene] OR 2,2-bis-(p-chlorophenyl)-1,1,1 trichloroethane
10. β -HCH (β -hexachlorocyclohexane - isomeric contaminant formed during manufacture of insecticide lindane) : (1 α ,2 β ,3 α , 4 β ,5 α ,6 β)-1,2,3,4,5,6-hexachloro-cyclohexane
11. Endosulfan (α -isomer) : (3 α ,5a β ,6 α ,9 α , 9a α)-6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro 3-oxide,6,9-methano-2,4,3,benzo-dioxathiepin
12. Endosulfan (β -isomer) : (3 α , 5a α , β , 9 β , 9a α)-3 oxide-6,7,8,9,10,10-hexachloro - 1,5,5a,6,9,9a-hexahydro-6,9, methano-2,4,3-benzodioxathiepin
13. o'p' DDE : 2 (o-chlorophenyl)-2-(p-chlorophenyl)1,1,di chloroethylene
14. p'p' DDE : 2,2 bis-(p-chloro phenyl)-1,1-dichlororethylene

• ORGANOCHLORINATED PESTICIDES

15. Aldrin (pesticide): (1 α , 4 α , 4a α , 5 α , 8 α , 8a α)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanophtalene. OR 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro -1,4:5,8 - dimethanonaphtalene (HHDN)
16. Benzophenone : (manufacture of insecticides and anti histamines; fixture in strong perfumes (soaps and shampoos)
17. Butylated hydroxytoluene (BHT) : (Anti oxidant in food packaging, PVC, cellulose and certain elastomers, and insect repellents)
18. Chlordane (pesticide): 4,7, methano-1-H-indene -1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-chlordane
19. Dieldrin (pesticide):(1a α , 2 β , 2a α , 3 β , 6 β , 6a α , 7 β , 7a α)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a,-octahydro-2,7,3,6 dimethanophtl[2,3b]oxirene OR 1,2,3,4,10,10 hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo OR exo-1,4:5,8 dimethanophtalene.
20. Lindane : (1 α , 2 α , 3 β , 4 α , 5 α , 6 β)-1,2,3,4,5,6-hexachloro-cyclohexane (gamma HCH) (broad spectrum insecticide used in a variety of agricultural, medical and veterinary products. In the USA it is a active component of a shampoo (Kwell) used for the treatment of pediculosis as well as for limited use on food crops)
21. Toxaphene (organochlorine pesticide)

• PCB's

1. Arochlor 1254 & 1260
2. PCB's (organochlorine compounds eg. dichlorodiphenyltrichloorthane and products of combustion eg. petrol engine) eg. 2'4'6'trichlor-4-bi-phenylol and 2'3'4'5'tetrachloro-4-biphenylol.
3. Some PCB's

• PLASTICISERS

1. BBP (plasticiser, especially in the production of vinyl floors): 1,2-benzenedicarboxylic acid, esters, butyl phenylmethyl-esters.
2. Bisphenol A (fungicide in polycarbonates) : 4,4'-(1-methylethylidene)bisphenol
3. DEHA : Bis-(2-ethyl-hexyl)adipate : (manufacture of plastics)
4. p-tert-Butylbenzoic acid (plastic industry; corrosion inhibitor in polystyrene manufacture in dyeing)
5. Phtalates: eg. n-butyl phtalate, diphenylphtalate, and ethylhexylphtalate
6. Di(2)ethylhexyl)phtlates (plasticider for PVC)
7. Diphenylphtalate
8. Plastics : bisphenol A
9. Polycarbonate plastics and waterjugs
10. Polysyrene tubing

• INDUSTRIAL

1. 3,9-Dihydroxybenz(a)anthracene (combustion product)
2. 4-Nitrotoluene (manufacture of dyes)
3. Corn oil and related poly unsaturated fats
4. Fuel constituents : e.g. aromatic hydrocarbons
5. n-Butylbenzene : (petrochemical origin)
6. p-Cresol (4-methylphenol) : to produce anti oxidant, UV stabilizer
7. Toluene (industrial solvent)
8. Paper Mill wastes: eg. chlorine bleaches
9. Phenol red

• PHARMACEUTICAL

1. Adiol (anabolic agent): 5-androstene-3- β -17- β -diol
2. Caffeine : (found in drinks and pharmaceuticals):
3. Cimetidine :N-cyano-N'-methyl-N''-[2-[[[(5-methyl-1H-imidazol-4-yl)methyl]thioethyl-guanidine OR N-cyano-N''-methyl-N''-[2-[[[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]guanidine, SKF
4. Clenbuterol : 4 amino-3,5-dichloro- α [[[(1,1-dimethylethyl-amino)methyl]-benzene-methanol OR 4-amino- α -[(tert-butylamino)methyl]-3,5-dichloro-benzyl alcohol ,NAB
5. Cycloheksimied : [1s-[1 α , (s*), 3 α , 5 β]]-4-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-2,6 piperidinedione (also a fungicide)
6. Diethylstilboestrol (DES)
7. Ginseng
8. Mifepristone (RU 486) : 11-[4-(dimethylamino)phenyl]-17-hydroxy-17-(1-propynyl)-(11 β , 17 β)-estra-4,9,dien-3-one OR (11 β , 17 β)-11-[4-(bi methylamino)-phenyl]-17-hydroxy-17-(1-propyl)estra-4,9-dien-3-one
9. o'p' DDD (anti neoplastic): 2 (o-chlorophenyl)-2-(p-chlorophenyl)-1,1-dichloroethylene
10. p-Hydroxy benzoic acid (cosmetic, food and pharmaceutical preservative)
11. Synthetic estrogens : ethinylestradiol
12. Prednisolone : 11,17,21, trihydroxy-(11 β)-pregna-1,4-diene-3,20-dione OR (11 β -11,17,21-trihydroxypregna-1,4-diene,3,20,-dione
13. Toremifene : (Z)-2-[4-(4-chlor-1,2-diphenyl-1-butenyl)phenol]-N,N-dimethyl-ethanamine
14. Pharmaceuticals : synthetic estrogens
cimetidine

• NATURALLY OCCURRING SUBSTANCES

1. Boron
2. Broccoli
3. Cholesterol : (excreted steroid, emulsifying agent)
4. Immounoglobulin G (IgG)
5. Milk from pregnant cows
6. Mycotoxins
7. Phytohormones
8. Resorcylic acid lactones : 2,4-dihydroxybenzoic acid
9. Xenobiotics
10. Xenoestrogens (foreign estrogens)

• NATURAL ESTROGENS

1. Enterodiol : 2,3-bis[(3-hydroxyphenyl)methyl]-1,4-butanediol
2. Estrone : steroidal estrogen 3-hydroxyestra 1,3,5(10) triene -17-one
3. Estradiol : 1,3,5(10)-estratriene,3,17- β -diol-estradiol OR (8 α ,9 β ,13 α ,14 β ,17 α)-estrs 1,3,5 (10)-trine-3,17-diol
4. Estrogen found in sewage water
5. Estrone : 3-dihydroxy-estra 1,3,5 (10)-triene-17-one
6. Steroidal estrogens

• PHENOLS

1. 2,2'-dihydroxy5'-dichlorophenylphenol
2. Alkyl phenolic compounds :
 - 4-octylphenol
 - 4-nonylphenol
 - 4-nonylphenol-di-ethoxylate
 - 4-nonylphenoxycarboxylic acid
3. APE (alkylphenolic ethoxylates)
4. APEC
5. APEO's : alkylphenolic poly ethoxylates eg. nonylphenolpolyethoxylates (80% of APEO's) & octylphenol poly ethoxylates (20% of APEO's)
6. Dichlorophenol (found in fungicide and germicide products)
7. Dimethylphenol (disinfectant /microbicide)
8. Pentachlorophenol
9. Alkyl Phenols : pentaphenol to nonyl phenol
10. BHA : (1,1-dimethylethyl)-4-methoxy-phenol
11. Nonylphenol
12. o' Phenylphenol
13. Octyl phenol

• PLANT SUBSTANCES

1. Anthocyanidins
2. Chalcones
3. Coumestrol (most potent plant isoflavonoid) : 3,9,dihydroxy-6H-Benzofuro
[3,2-c][1]benzopyran-6-one OR 3'5'-dihydroxy-coumestane OR 2-(2,4 -
dihydroxyphenyl)-6-hydroxy-3-benzofurancarboxylic acid- gamma-lactone
4. Enterolactone : trans, dihydro-3,4-bis[3-hydroxyphenyl)methyl]-2(3H)-furanone
5. Equol (phytoestrogen) : (s)-3,4 di hydro-3-(4-hydroxyphenyl)-2H-1-benzopyran-7-ol
6. Flanonols (3-hydroxyflavnes)
7. Flavones : 3,hydroxy-2-phenyl-4H-1-Benzopyran-4-one
8. Flavonones
9. Isoflavones (genistein, daidzein, formononetin)
10. Isoflavones : 2,3-dihydro-3-phenyl-4H-1-Benzopyran-4-one
11. Phloretin : 1-proponone-3-(4-hydroxyphenyl)-1-(2,4,6 trihydroxyphenyl-phloretin OR
2',4,4',6'-tetrahydroxydihydrochalone.(phloretin) OR 3-(4-hydroxy phenyl)-
1(2,4,6 tri hydroxy-phenyl)-1-propone OR 2'4'6'trihydroxy-3-(4-hydroxy-
phenyl)propiophenone
12. Phytoestrogens [occurs naturally in foods such as soybeans, whole wheat and flaxseeds . Plants
containing compounds with estrogen activity eg. soy contains diazin, wheat
contains enterolactones (less potent)]
coumestrol
genistein : 4'5,7-trihydroxy-isoflavone OR 5,7 dihydroxy-3-(4-hydroxyphenyl-
4H-1-benzo-pyran-4-one
salsolidine : 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline
hydrochloride
salsoline : 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroxy-1,2,3,4-tetrahydro -
isoquinoline bromide
salsolinol : 1-methyl-6-hydroxy-1,2,3,4-tetrahydroxy isoquinoline hydrochloride
13. Ricinus communis var. minor (seed)
14. Soyabeans
15. Zearelenone (:3,4,5,6,9,10,11,12,-octahydro-14,16-dihydroxy-3-methyl-(s)-1H-2-
benzoxacyclotetradecin-1,7-(8H)-dione
16. Alliquotes of the extracts tested for total estrogen activity in Medicago Sativa (α - α plant)
17. Soy

• MISCELLANEOUS

1. 1-Hydroxychlordane
2. Benzo[a]pyrene (combustion byproduct of foods and cigarettes) : 3,4 benzpyrene
3. DDE (a metabolite of DDT, a contaminant in dicofol, a insecticide sprayed on food crops): 1,1'-(dichloroethenylidene)bis [4-chloro-benzene]
4. Dicofol (acaricide): 4-chloro- α -(4-chlorophenyl)- α -(trichloromethyl)-benzene-methanol
5. PAH (polycyclic aromatic hydrocarbons) (inhibitor of dihydrofolatereductase) : N-(4-aminobenzoyl)-glutamic acid
6. Tetrahydro-isoquinolines (TIQ) : (research tool in neurobiological biology)
Tetrahydropaperoline : [1-(3'4'dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrobromide OR 1-[(3,4-dihydroxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-isoquinolinediol OR 1-(3,4-dihydroxybenzyl)-1,2,3,4-tetrahydro-6,7-isoquinolinediol
7. PDDF's: dibenzofurans
8. (1 α , 2 β , 2 α , 3 β , 6 β , 6 α , 7 β , 7 α)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7,3,6-dimethanoaph-[2,3-b]oxirene
9. 3 Adihydroxybeza[a]-anthracene
10. Aromatic Hydrocarbons
11. Carboxylic acid
12. Chicken manure
13. Chlorinated hydrocarbons
14. Chlorinated organic compounds: Atrazine
Chlordane
DDT
Endosulfan
Kepone
Methoxychlor
Some PCB's
15. Chlorine compounds that bleach paper
16. Clone ID5 : anti-idiotypic antibody
17. Crops grown in animal pastures
18. DBP (dispersant in insect repellents, and found in plastic plumbing pipes and foodwraps) : bis (4-chlorophenyl)-methanonone
19. DEHP : Bis (ethyl-hexyl)phtalate) (source : plasticizer)
20. DMBA : 7,12 di methyl benz (a) anthracene
21. Epidermal Growth Factor (human) : 3L-aspartic acid-4-L-serine-5L-glutamic acid-8L-leucin
10L-histidine-16L-histidine-17L-aspartic acid-22L-tyrosine- 25L-alanine-28L-lysine-30L-alanine-35L-valine-38L- isoleusine-40L- glutamicacid-44L-tyrisine-48L-lysine- α - epidermal growth factor.
22. Manure of swine, goats, horses, cows
23. Milk
24. Musk ketone (scent)
25. Musk xylene (scent)
26. Organochlorines
27. PCDD : Poly chlorinated dibenzo-p-dioxins
28. Petroleum by-products
29. Some petroleum products
30. TCDD : 2,3,7,8 tetra chloro-dibenzo [be]-[1,4]-dioxin
31. TCDD-EQ : 2,3,7,8,-tetrachlorodibenzo-p-dioxins equivalents

Appendix 2 Classification and uses of estrogen and estrogen-mimics

	<i>Estrogen / estrogen mimic</i>	<i>Source</i>	<i>Usage</i>
Classification			
Herbicides	2,4 D	(Raloff, 1994:56-58)	Broadleaf herbicide used in North America
	Atrazine	(Begley & Glick, 1994:44; Lee Davis & Bradlow, 1995:144-149; Raloff, 1993:10-15 & Warhurst, 1995a:756)	Triazine herbicide
	Dibenzodioxin-2,3,7,8-tetrachlorodioxin	(Rennie, 1993:15)	Herbicidal contaminant
	Methoxychlor	(Hileman, 1994:19-23; Lee Davis & Bradlow, 1995:144-149; & Thomas <i>et al.</i> , 1993:147-151)	Herbicide
	Methylphenol	(Geibler & Scholer, 1994:2047-2053)	Found in herbicide products / phenolic resins
Pesticides / Insecticides	Chlordecone (Kepone, CD)	(Soto <i>et al.</i> , 1994:380-384)	Insecticide
	DDT	(Lee Davis & Bradlow, 1995:144-149; Raloff, 1994:56-58 & Rennie, 1993:15)	Insecticide
	Endosulfan	(Begley & Glick, 1994:44; Melius, 1995:77-79; Raloff, 1994:56-58 & Soto <i>et al.</i> , 1994:380-384)	Insecticide
	Heptachlor	(Raloff, 1984:24-27 & Raloff, 1993:10-15)	Insecticide for the control of cotton boll weevil
	Kepone	(Field <i>et al.</i> , 1990:4-54; Hileman, 1994:19-23:19-23; Rennie, 1993:15 ; Lee Davis & Bradlow, 1995:144-149; Thomas <i>et al.</i> , 1993:147-151; Raloff, 1984:24-27)	
	Malathion	(Dutta <i>et al.</i> , 1994:215-220)	Insecticide
	Mirex	(Hansen & Jansen, 1994:525-527 & Raloff, 1984:24-27)	Insecticide, rife retardant for plastics, rubber, paper and electrical goods

Classification	Estrogen / estrogen mimic	Source	Usage
Pesticides / insecticides (continued)	p'p' DDT	(ANON., 1995D:575; ANON., 1995D:575; Bustos <i>et al.</i> , 1988:496-502; Hileman, 1994:19-23; Mestel, 1994:9:9; Nelson <i>et al.</i> , 1978:237-246; Dibb, 1995:27- 31; Warhurst, 1995b:92a:756; & ANON., 1995D:575)	Insecticide
	o'p' DDT	(ANON., 1995D:575; ANON., 1995D:575; Dibb, 1995:27-31; Forster <i>et al.</i> , 1975:1777-1778; Soto <i>et al.</i> , 1994:380- 384; Hileman, 1994:19-23; Nelson <i>et al.</i> , 1978:325-341; Raloff, 1984:24-27; Raloff, 1993:10-15 & Warhurst, 1995b)	Insecticide
	β-Hexachlorocyclohexane (β-HCH) / bensenehexa- chloride	(Fingler <i>et al.</i> , 1994:1135-1140)	Isomeric contaminant formed during manufacture of the insecticide lindane
	Endosulfan (alpha isomer)	(Begley & Glick, 1994:44; Melius, 1995:77-79; Raloff, 1994:56-58 & Soto <i>et al.</i> , 1994:380-384)	Insecticide
	Endosulfan (beta isomer)	(Melius, 1995:77-79; Raloff, 1994:56-58; Begley & Glick, 1994:44 & Soto <i>et al.</i> , 1994:380-384)	Insecticide
	o'p' DDE	(Raloff, 1984:24-27)	Insecticide
	p'p' DDE	(Gills, 1994:296-298 & Nelson <i>et al.</i> , 1978:352-341)	Insecticide
		(Gills, 1994:296-298 & Nelson <i>et al.</i> , 1978:352-341)	Insecticide

	<i>Estrogen / estrogen mimic</i>	<i>Source</i>	<i>Usage</i>
Classification			
Organochlorinated pesticides	Aldrin	(ANON., 1985: 287 & Scafferner <i>et al.</i> , 1983)	Pesticide
	Benzophenone		Manufacture of insecticides and antihistamines
			also used as a fixtue of strong perfumes
	Butylated hydroxytoluene (BHT)		Anti oxidant in food packaging, PVC, cellulsics
			and certain elastomers and insect repellents
	Chlordane	(Lee Davis & Bradlow, 1995:144-149; Scafferner <i>et al.</i> , 1983 & Soto <i>et al.</i> , 1992:295-309)	Pesticide
	Dieldrin	(ANON., 1985:218; Begley & Glick, 1994:44 & Melius, 1995:77-79; Raloff, 1984:24-27; Raloff, 1994:56-58;Scafferner <i>et al.</i> , 1983; & Soto <i>et al.</i> , 1994:295-309)	Pesticide
	Lindane (gamma BHC)	(Warhurst, 1995b:92)	Broad spectrum insectiside used in agricultural
			medical & veterinary products also a component
			in pediculosis shampoo Kwell
	Toxaphene	(Melius, 1995:77-79; Raloff, 1984:24-27; Raloff, 1994:56-58 & Soto <i>et al.</i> , 1994:380-384)	Organochlorine pesticide

	Estrogen / estrogen mimic	Source	Usage
Classification			
Polychlorinated biphenyls	Arochlor 1254	(Safe, 1994:87-149 & Green <i>et al.</i> , 1975:507-510)	
(PCB's)	Arochlor 1260	(Roux <i>et al.</i> , 1994:333-340; Safe, 1994:87-149 & Roux <i>et al.</i> , 1994:333-340)	
	PCB's e.g. organochlorine compounds like: 2'4'6' tri chlor-4-bipehnylol 2'3'4'5' tetrachloro-4-biphenylol dichlorophenyltrichloorethane	(ANON., 1995D:575; Ginsburg, 1994:284-285; Lee Davis & Bradlow, 1995:144-149; Raloff, 1994:56-58; Sager <i>et al.</i> , 1991:737-746; Scafferner <i>et al.</i> , 1983; ANON., 1995D:575; Soto <i>et al.</i> , 1994:380-384; Stone, 1992:308-310; Warhurst, 1995b:92a: & Weiss, 1994:169-175:169-175)	Used as dielectrical fluids in transformers heat transfer liquids to textile coatings, pesticide extenders as well as in kiss proof lipstick
	Some PCB's	(Lee Davis & Bradlow, 1995:144-149; Raloff, 1984:24-27; Sager & Girard, 1994:52-72 & Hileman, 1994:19-23)	
Plasticisers	BBP	(ANON., 1995D:575)	Plastiziser, especially in the production of vinyl floors
	Bisphenol A	(Hileman, 1994:19-23; Lee Davis & Bradlow, 1995:144-149 & Warhurst, 1995b:92a:)	Fungicide in polycarbonates, also used to coat metal containers such as foodtins
	Bis(2-ethylhexyl)adipate (DEHA)		Used in the manufacture of plastics
	p-tert-Butylbenzoic acid		Used in the plastic industry, corrosion inhibitor in polystyrene manufacture in dyeing
	Phtalates e.g. n-butyl phtalate	(ANON., 1995D:575; Hansen & Jansen, 1994:525-527 & Warhurst, 1995b:92)	Widely used as plasticisers
	diphenylphtalate	(ANON., 1995D:575)	
	ethylhexylphtalate		
	Di(2)ethylhexyl phtalates	(ANON., 1995D:575)	Plasticiser for PVC

Classification	Estrogen / estrogen mimic	Source	Usage
Plasticisers (continued)	Plastics	(Lee Davis & Bradlow, 1995:144-149)	
	Polycarbonate plastics and waterjugs	(Begley & Glick, 1994:44)	
	Polystyrene tubing	(Raloff, 1993:10-15)	
Industrial	3,9 - Dihydroxybenz(a)anthracene	(Raloff, 1993:10-15)	Combustion product
	4-Nitrotoluene		Manufacture of dyes
	n-Butylbenzene		Petrochemical origin
	p-Cresol		Used to produce antioxidant. UV stabilizer
	Toluene		Industrial solvent
	Paper mill wastes e.g. chlorine bleaches	(Raloff, 1995:44-46)	Used in the paper manufacturing industries
	Corn oil and related poly unsaturated fats	(Lee Davis & Bradlow, 1995:144-149)	Used in the food industries
	Fuel constituents : aromatic hydrocarbons	(Lee Davis & Bradlow, 1995:144-149)	
	Phenol red	(Ernst <i>et al.</i> , 1989:907-914)	
Phenols	2,2'dihydroxy 5' dichlorophenylphenol	(Wood & Richardson, 1978:491-496)	
	Alkylphenolic compounds: e.g. 4-octylphenol	(Mestel, 1994:9:9)	Industrial detergents
	4-nonylphenol	(Hileman, 1994:19-23:19-23 & Ginsburg, 1994:284-285; Raloff, 1984:24-27 & Scafferner <i>et al.</i> , 1983)	Used grossly in the textile and pulp paper industries
	4-nonylphenol-di-ethoxylates		
	4-nonylphenoxycarboxylic acid		
	Alkylphenolic ethoxylates (APE's)	(ANON., 1995D:575; Purdom <i>et al.</i> , 1994:275-285 & Scafferner <i>et al.</i> , 1983)	Used as liquid clothes detergents in the USA
	Alkylphenolic poly ethoxylates (APEO's) e.g. nonylphenolpolyethoxylates octylphenolpolyethoxylates	(ANON., 1995D:575; Ginsburg, 1994:284-285 & White <i>et al.</i> , 1994:175-182)	

	<i>Estrogen / estrogen mimic</i>	<i>Source</i>	<i>Usage</i>
Classification			
Phenols (continued)	APEC	(White <i>et al.</i> , 1994:175-182)	
	Dichlorophenol		Found in fungicide and germicide products
	Dimethylphenol		Disinfectant / microbicide
	Pentachlorofenol	(Wood & Richardson, 1978:491-496)	
	Alkyl phenols : pentaphenol to nonylphenol	(Hileman, 1994:19-23; Scafferner <i>et al.</i> , 1983 & Warhurst, 1995b:92a)	
	1,1, dimethylethyl-4-methoxy-phenol (BHA)	(ANON., 1995D:575)	
	Nonylphenol	(Cotton, 1994:414; Lee Davis & Bradlow, 1995:144-149; Purdom <i>et al.</i> , 1994:275-285 & Weiss, 1994:169-175)	
	o' Phenylphenol	(Bicknell <i>et al.</i> , 1995:7-9 & Wood & Richardson, 1978:491-196)	
	Octylphenol	(Bicknell <i>et al.</i> , 1995:7-9 & Hileman, 1994:19-23)	
Plant substances containing estrogen	Anthocyanidins	(Miksicek, 1993:37-43)	
	Chalones	(Miksicek, 1993:37-43)	
	Coumestrol	(Miksicek, 1993:37-43; Shore <i>et al.</i> , 1992:217-222; Soto <i>et al.</i> , 1992:295-309 & Whitten <i>et al.</i> , 1995:771s-777s)	
	Enterolactone	(Welshons <i>et al.</i> , 1987:169-175)	
	Equol	(Welshons <i>et al.</i> , 1987:169-175)	
	Flavonols	(Miksicek, 1993:37-43)	
	Flavones	(Miksicek, 1993:37-43)	
	Flavonones	(Miksicek, 1993:37-43)	
	Isoflavones	(Miksicek, 1993:37-43)	
	Phlorethin	(Miksicek, 1993:37-43)	

Classification	Estrogen / estrogen mimic	Source	Usage
Plant substances containing estrogen (continued)	Phytoestrogens e.g. coumestrol	(ANON., 1995D:575; Field <i>et al.</i> , 1990:44-54; Hileman, 1994:19-23 & Larkin, 1995:144-149)	Occurs in naturally in foods such as soybeans,
	genistein		whole wheat, and flaxseeds
	salsolidine		
	salsoline		
	salsolinol		
	Ricinus communaris var. minor (seed)		
	Soyabeans	(Vines, 1993:5:5)	Used as a protein substitute for vegetarians
	Soy	(Ginsburg, 1994:284-285)	Used as a protein source in vegetarian meals and in
			baby milk
	Zearelenone	(Shoental, 1985:15)	
	Alliquotes of the extracts tested for total estrogen		
	content of the Medicago Sativa plant (alfalfa plant)	(Okwuasaba <i>et al.</i> , 1991:141-145 & Shore <i>et al.</i> , 1992:217-222)	

	Estrogen / estrogen mimic	Source	Usage
Classification			
Miscellaneous	1-Hydroxychlordene		
	Benzo(a)pyrene	(Weiss, 1994:169-175)	Combustion byproduct of foods and cigarettes
	DDE	(Dibb, 1995:27-31; Hileman, 1994:19-23; Raloff, 1984:24-27; Raloff, 1995:44-46; Taubes, 1994:499-500 & Weiss, 1994:169-175)	The metabolite of DDT. A contaminant in dicofol. A
			insecticide sprayed on food crops
	Dicofol	(Hileman, 1994:19-23 & Raloff, 1984:24-27)	Acaricide
	Tetrahydro-isoquinolines (TIQ)	(Stammel <i>et al.</i> , 1991:1319-1330)	Research tool in neurobiological biology
	PDDF's		
	Estrogens found in sewage water	(Shore <i>et al.</i> , 1992:217-222)	
	(1,2,2,3,6,7,7)-hexachloro-1,2,2,3,6,6,7,7-octahydro-2,7,3,6-dimethanoaph-2,3-oxirene		
	Poly aromatic hydrocarbons (PAH's) e.g. Anthracene Benz(a)anthracene Dibenz(a,h)anthracene 3,9 Dihydroxybenz(a)anthracene 3 adihydroxybenz(a)anthracene Dimethylbenz(a)anthracene (DMBA)	(Field <i>et al.</i> , 1990:44-54 & Raloff, 1993:10-15)	Inhibitor of dehydrofolatereductase Combustion product

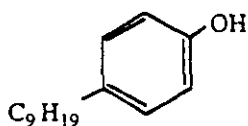
	Estrogen / estrogen mimic	Source	Usage
Classification			
Miscellaneous (continued)	3 adihydroxybenz(a)anthracene	(Hileman, 1994:19-23)	
	Aromatic hydrocarbons	(Giesy <i>et al.</i> , 1994:128-135; Poland & Knutson, 1982:517-554 & Zoller, 1993:187-194)	
	Carboxylic acid	(White <i>et al.</i> , 1994:175-182)	Used to carbonate cooldrinks
	Chicken manure	(Shore <i>et al.</i> , 1993:361-366)	Fertilisers for lawn
	Chlorinated hydrocarbons	(Dibb, 1995:27-31)	
	Chlorinated organic compounds e.g. atrazine		
	chlordane		
	DDT		
	endosulfan		
	kepone	(Field <i>et al.</i> , 1990:44-54)	
	methoxychlor		
	some PCB's		
	Chlorine compounds that bleach paper	(Begley & Glick, 1994:44)	Used in the paper manufacturing industry
	Clone ID5	(Mor <i>et al.</i> , 1992:3633-3640)	
	Crops grown in animal pastures	(Wilcox & Wahlquist, 1990:905-906)	
	Bis (4-chlorophenyl)-methanonone (DBP)	(ANON., 1995D:575)	Dispersant in insecticide repellents, found in plastic plumbingppypes and foodwraps
	Bis (ethylhexyl)phtalate (DEHP)		Source : plasticizer
	Dimethylbenz(a)anthracene (DMBA)	(Pasqualini <i>et al.</i> , 1988:6434-6437)	
	Epidermal growth factor (human)	(Ignar - Trowbridge <i>et al.</i> , 1992:4658-4662)	
	Manure of swine, horses, goats, and cows	(Shore <i>et al.</i> , 1993:361-366)	Fertiliser for lawn
	Milk	(Shore <i>et al.</i> , 1993:361-366 & Vines, 1993:5)	Human food additive
	Tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQ)		
	Tetra chloro-dibenzo-dioxins (TCDD)	(ANON., 1995D:575; Malby <i>et al.</i> , 1992:18-126; Raloff, 1995:44-46; Silverstone <i>et al.</i> , 1992:103-106 & Zacharewski <i>et al.</i> , 1995:2140-2416)	Produced during the incineration e.g. the production of chlorinated hydrocarbons and paper production.

	<i>Estrogen / estrogen mimic</i>	<i>Source</i>	<i>Usage</i>
Classification			
Miscellaneous (continued)	Petroleum by-products	(Raloff, 1993:10-15 & Rennie, 1993:15)	
	Poly chlorinated dibenzo-p-dioxins (PCDD)	(Dibb, 1995:27-31 & Raloff, 1995:44-46)	Dioxins are unintentional by-products of pesticide processing. They are also present as contaminants in pesticides, and are found in technical products, emissions from municipal, hospital and industrial waste incineration and materials associated with bleaching pulp and other uses of chlorine.
	Musk ketone (scent)		
	Musk xylene (scent)		
	Organochlorines	(ANON., 1985:218)	
Pharmaceuticals	Adiol	(Leroy <i>et al.</i> , 1988:453-458 & Mueller & Kim, 1978:1429-1435)	Anabolic agent
	Caffeine		Found commonly in drinks and pharmaceuticals
	Cimetidine	(Lee Davis & Bradlow, 1995:144-149)	Used in the treatment of stomach ulcers
	Clenbuterol	(Perez <i>et al.</i> , 1993:233-243 & Re <i>et al.</i> , 1993:438-442)	Used in asthma treatment
	Cyclohexymide	(Perez <i>et al.</i> , 1993:233-243)	Used as a fungicide
	Ginseng	(Hopkins <i>et al.</i> , 1988:1112-1122)	Vitamine supplement
	Mifepristone (RU 486)	(Dibbs <i>et al.</i> , 1995:134-140)	
	o'p' DDD	(ANON., 1993b:11)	Anti neoplastic agent
	p-Hydroxy benzoic acid		Use in cosmetics, foods and pharmaceutical preservatives
	Synthetic estrogens e.g. Diethylstilboestrol	(Aherne & Briggs, 1989:735-136; Lee Davis & Bradlow, 1995:144-149; ANON., 1995D:575; Field <i>et al.</i> , 1990:44-54 & Vines, 1993:5:5)	Oral contraceptive

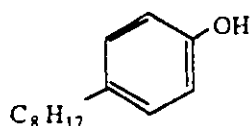
	<i>Estrogen / estrogen mimic</i>	<i>Source</i>	<i>Usage</i>
Classification			
Pharmaceuticals (continued)	Ethinylestradiol	(Aherne & Briggs, 1989:735-136; De Vito <i>et al.</i> , 1991:321-338; Dibb, 1995:27-31; Purdom <i>et al.</i> , 1994:275-28 & Raloff, 1984:24-27)	Oral contraceptive, acne treatment
	Prednisolone	(Mahli <i>et al.</i> , 1991:690-693)	Used in asthma treatment, corticosteroid
	EPA (5,8,11,14,17 - Eicopentaenoic acid)	(Budavari <i>et al.</i> , 1989:3499)	Used for antihyperlipoproteinemia
Naturally occurring substances	Boron	(Nielsen <i>et al.</i> , 1992:237-246)	Vitamine supplement
	Broccoli	(ANON., 1994B:414-416)	Vegetable
	Cholesterol	(Moore <i>et al.</i> , 1991:85-97)	Emulsifying agent
	Immunoglobulin G (IgG)	(Borkowski <i>et al.</i> , 1991:3238-3292)	
	Milk from pregnant cows	(Perez <i>et al.</i> , 1993:233-243 & Vines, 1993:5.5)	Food substance for human consumption
	Xenobiotics	(Palmer & Palmer, 1995:19-25; Soto <i>et al.</i> , 1991:167-173 ; Soto <i>et al.</i> , 1992:295-309 & Thomas <i>et al.</i> , 1993:147-151)	
	Xenoestrogens (foreign estrogens)	(Lee Davis & Bradlow, 1995:144-149)	
	Resorcylic acid lactones	(Wilcox & Wahlquist, 1990:905-906)	Reproductive hormones produced by the genus <i>Fusarium</i> and are commonly found contaminants of improperly stored grain.
	Mycotoxins	(Li <i>et al.</i> , 1985:168-183; Shoental, 1985:15 & Soto <i>et al.</i> , 1992:295-309)	
Endogenous estrogens	Enterodiol	(Hileman, 1994:19-23; Shore <i>et al.</i> , 1993:361-366 & Welshons <i>et al.</i> , 1987:169-175)	
	Estrone	(Shore <i>et al.</i> , 1993:361-366)	
	Estradiol	(Mor <i>et al.</i> , 1992:3633-36410 & Meikle <i>et al.</i> , 1994:191-202 & Mueller & Kim, 1978:1429-1435:1429-1435)	

Appendix 3 Structures of estrogen and estrogen - mimics

Alkylphenolic compounds

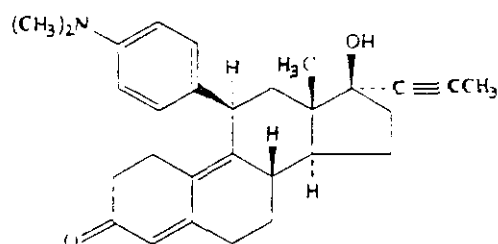


Nonylphenol

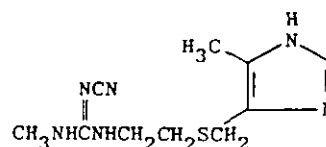


4-Octylphenol

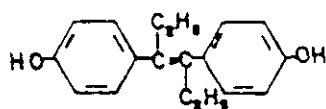
Pharmaceutical products that mimic estrogen



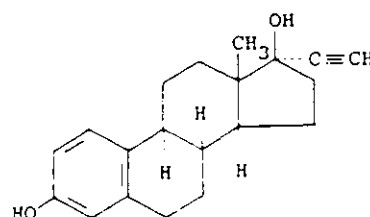
Mifepristone



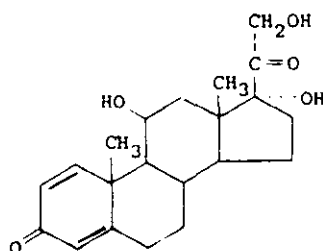
Cimetidine



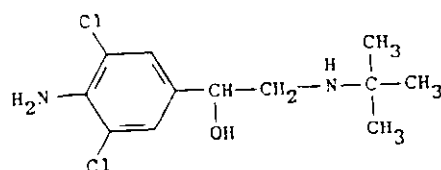
Diethylstilboestrol



Ethinylestradiol

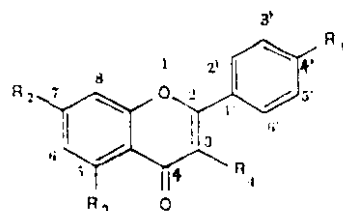


Prednisolone

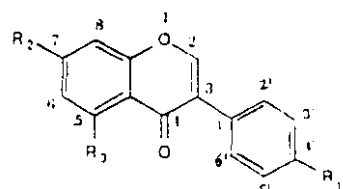


Clenbuterol

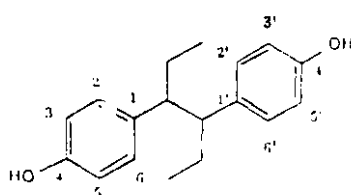
Phytoestrogens



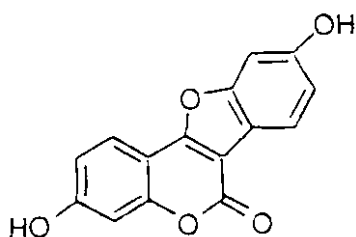
Flavones	R1	R2	R3	R4
4',5,7-trihydroxy (Apigenin)	-OH	-OH	-OH	
3,4',5,7-tetrahydroxy (Kaempferol)	-OH	-OH	-OH	-OH



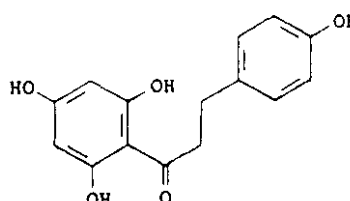
Isoflavones	R1	R2	R3
4',5,7-trihydroxy (Genistein)	-OH	-OH	-OH



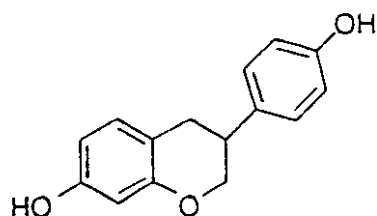
Dihydroxy Stilbenes	Bridge Conjugation
Hexestrol	CH ₃ -CH ₂ -CH=CH-CH ₂ -CH ₃
Diethylstilbestrol	CH ₃ -CH ₂ -C≡C-CH ₂ -CH ₃



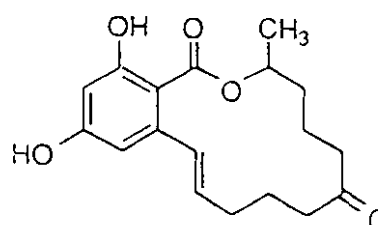
Coumestrol



Phloretin

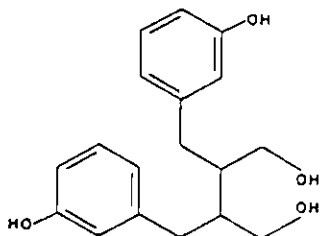


Equol

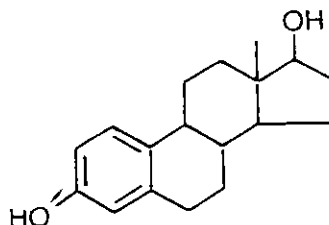


Zearalenone

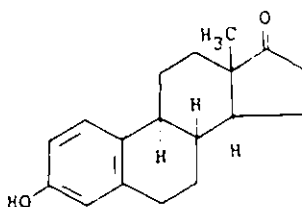
Endogenous estrogens



Enterodiol

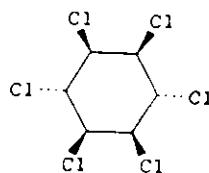


β - Estradiol



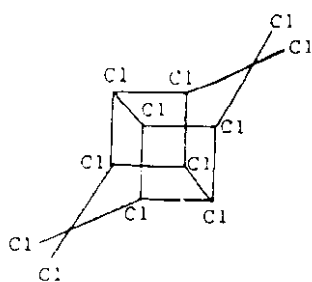
Estrone

Organochlorinated pesticides

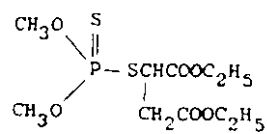


Lindane

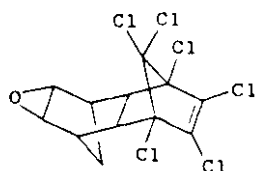
Other pesticides / insecticides



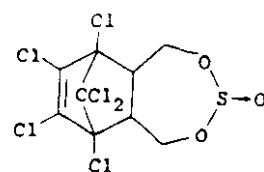
Mirex



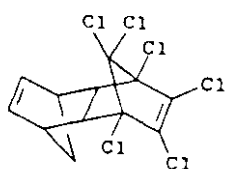
Malathion



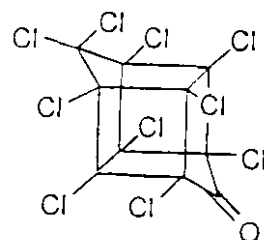
Dieldrin



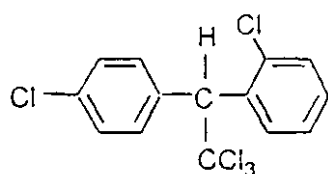
Endosulfan



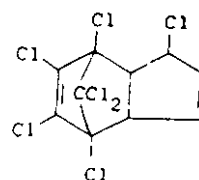
Aldrin



Kepone

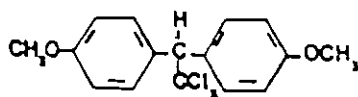


o'p' DDT



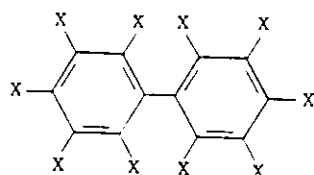
Heptachlor

Herbicides



Methoxychlor

Polychlorinated biphenyls (PCB's)



X = H or Cl

An general structure of PCB's

Appendix 4 Institutions contacted for the tracing of estrogen and estrogen-mimics in the South African water environment

Appendix 4a Institutions who received faxes

- Universities and Technicons :

Dr D.J. Steyn : Rand Afrikaans University, Dept. Zoölogy
Dr J. Day : University of Cape Town, Dept. Fresh Water Institute
Dr J.H. van Wyk : University Stellenbosch, Dept. Zoölogy
Dr L. London : University of Cape Town, Dept. Community Health
Dr P. van Rossum : retired from the University of Pretoria
Dr Spark : Technicon Natal, Dept.. Chemical Sciences
Mr M. Laing : University of Natal, Dept. Microbiology
Mr V. Wepener : University of Zoeloeland, Dept. Zoölogy
Mrs A. van der Merwe : University of Pretoria :Dept. Chemical Pathology
Prof Tereblanche : University of Zoeloeland, Dept. Biochemistry & Microbiology
Prof W. Grabow : University of Pretoria, Dept. Virology

- State departments :

Mr L. Bredenhann : Dept. Water & Forestry (Pta)
Mr D. Grobler : Institute for Water Quality Studies (Pta)
Mr D. Whyte : CSIR, Watertech
Miss L. Slabbert : Dept. Water & Forestry (Pta)
Mrs A. Theunissen : ITSC
Mr W.L. Reichert : Benede Oranje District
Mr C. Arcibald : CSIR Watettech (Durban)
Miss B. Conradie : Dept. of Water & Forestry : Uppington
Prof Hodgson : Institute for Ground Water Studies
Mrs A. Burger : CSIR : FOODTECH (Pta)
Mr P. van Zyl : SABS : Dept. Residu Chemistry
Mrs H. Badenhorst : Dept. Water Affairs & Forestry
Dr P. Kempster : Institute for Water Quality Studies (Pta)
Mr A. Looke : Agricultural Research Council
Mr J du Plessis : Water Research Commission
Dr L. Lötter : Chemical & Allied Industries
Mr M. Keet : Dept. of Health (Pta)
Dr Walwyn : Dept. of Trade and Industries

- Laboratories :

Mr C. Schoeman : Cydna Laboratories
Mr D. Waddicar : Durban Waste Water Managment Laboratory
Mr Thirion : Water Lab (Pta)

- Industries :

Dr W. van Niekerk : AEC, Pretoria

Mr L. Hartzenberg : Karbochem

Mr J. Cooks : Karbochem

Mr F. Goede : SASTECH

Mr J. Venter : Naschem

Mr T. Snyman : NCS

Mr P. Foulds : Polifin

- Municipalities and Water Boards :

Mrs N. Basson : Goldfield Water

Mrs S. Jackson : Durban Municipality

Mr D.R. Thomelang : Bloemwater Board

Mrs S. Dark : CapeTown Engineer's Dept.

Mr W.N. Richards: Umgeni Water

- Pharmaceutical companies :

Mr R. Wood : Rhone-Poulenc

Appendix 4b Institutions contacted by phone

Institutions contacted for tracing of estrogen and estrogen-mimics in the South African water environment

Date	Contact person	Organization	Tel. no.	Fax no.	Referred to:	Organization	Reference(s)	Ref. organization	Ref. tel. no.	Remarks	Response	Contact updated	Response of update call
96/08/08	Mr J. Cooke	Karbochem	016-7951610	016-7951880	Mr P. Venter	Karbochem				Uses some of the substances in mixtures	Yes	27/08/96	Tel. response 17/09/96
96/02/08	Mr Rymaker	Natref	016-7992390								No		
96/02/08	Mr P. Foulds	Polifin	016-7932323	016-7932325							Yes		
96/05/08	Mr P. Symman	NCS	031-7016700	031-7990818	Passed on	Centrachem					No	27/08/96	Tel. response 5/08/96
10/07/96	Dr. Walwyn	Dept. of Trade & Industries	012-3109764	012-3220298							No		
96/01/08	Mr W. van der Merwe	Dept. of Health	012-3120000								No		
96/02/08	Mr E. Wood	Rhine Pollens	012-5611605	012-5611605			Dr P. Kempster				No		Sent DWAF documents
96/02/08	Mr A. Lonke	Agrie Res. Council	012-3264205	012-3231157			Plant Protection Res. Institute		012-8080952		No		Received fax
96/01/08	Dr L. Lotter	Chemical Allied Industries	011-4821671	011-7248710	Mr C. Schoeman	Cydia Laboratories			011-7287373		No	29/08/96	
96/02/08	Mrs H. Badenhorst	Dept. Water Affairs	012-8080777	012-8080738							Yes	27/09/96	Received fax 17/09/96
96/01/08	Mrs E. Gombault	Wastech	011-4565430								No		
29/08/96	Mr B. C. Nell	Municipality Potchefstroom	0148-2947744								No		
29/08/96	Mr D. Naude	Municipality Heilbron	05899-22014				Mr J. Pieterse	West Transvaal Waterboard	018-4824241		No		
29/08/96	Mrs S. Jackson	Municipality Durban	031-3024911	031-3024747			Mr J. E. Liebenberg	Wastech CSIR	051-4486250		No		
29/08/96	Mrs J. de Lou	Municipality Port Elizabeth	041-5063111				Prof. Oelofsen	UPE Biochemistry		Works on pesticide analysis	Yes		Received fax
29/08/96	Mr L. de Wet	Municipality Pretoria	012-3087911				Mrs C. Brand		8228075733	Does work on tralometane	No		
28/08/96	Mr Homan	Municipality Sasol	016-760029				Van der Byl Laboratories		016-3333333		No		
29/08/96	Mr S. Pieterse	Municipality Cape Town	021-6379090		Mrs S. Dark	Municipality CT	Mr van Vuuren	Vandoc	016-549821		No		
29/08/96	Mr Benade	Municipality Bloemfontein	051-4058911								Yes		Received copy of fax
96/02/08	Dr V. Hammett - Altwall	Naschem	0148-2977151	0148-2981148	Mr J. Venter	Naschem	Dr H. Bouman				Yes		
96/02/08	Mrs K. van der Walt	Potchefstroomse Universiteit vir CHO Biochemistry	0148-2992311				Prof. van Aardt	PU vir CHO Zoology			No		
96/01/08	Dr M. Lighthelm	Dept. Water & Forestry	012-2992482	012-8080338	Dr P. Kempster	Institute for Water Quality Studies	CSIR Wastech				No		
96/01/08	Dr O. Fourie	Olden Fount Toxicologists	012-3487732	012-3487416							No		
96/02/08	Mr J. de Plessis	Water Research Commission	012-3303040	012-3312565	Mrs A. Oelofsen	WRC					Yes		Received fax
96/02/08	Prof. W. van Aardt	Potchefstroomse Universiteit vir CHO Zoology	0148-2992376								No		
96/01/08	Mrs J. McCourt	SARS	012-4287911				Mr M. McInerney	CSIR Biotechnology	012-4286844	Done studies on heavy metals	No		
96/02/08	Prof. N. Nielsen	Potchefstroomse Universiteit vir CHO Chemistry	0148-2992304				Mr P. van Zyl	SARS Residue Division	012-4286446		No		
96/01/08	Dr B. Ray	Umgwen Water	0331-411111	0331-411177	Mr W.N. Richards	Umgwen Water Board	Mr B.C. Nell	Municipality Potchefstroom			No		
96/07/08	Mrs A. Burger	CSIR FOODTECH	012-8414214	012-8413594							Yes	30/09/96	Received fax
96/07/08	Mr D. Whyte	CSIR Environmental	012-8412232								Yes	27/08/96	
96/04/08	Mr D.R. Thonemang	Bloem Water Board	051-4472384				Dr K. van Niekerk	Food division	012-8413172	Done analysis on PCB's and nonylphenol	No	19/09/96	
96/04/08	Mr W. Vermeulen	Northern Transvaal Water	01524-86823	051-4479652	Mr L. van Outhoorn	Chief Technical Services					No	19/09/96	
96/04/08	Mr S. Milner	Goldfields of SA	011-6399111				Mr P. Grobler			Wants to help with the analysis	No		
96/07/08	Mr J. Koster	Magpies Water	0146-55809								No		
96/07/08	Mr F. Goede	SARTECH	016-7082908	016-7082826			Mr L. Meyer		01466-55884		No		
19/08/96	Mr S.A. Pieterse	Municipality of Cape Town	021-6379090	021-5385083	Mrs S. Dark	Municipality of Cape Town (Lab)				Scheduled a meeting July 1996	No	27/08/96	
29/08/96	Infotek		012-3422981							Does analysis on 16 pesticides	No	27/08/96	
30/08/96	Mr E. Wydenman		05391-3772				Mr G. Spanenberg	Gerhard Spanenberg Laboratories	05373-22690		No		
30/08/96	EVN Advisory Engineers		0148-2977305					Strata Laboratories	018-4622089		No		
30/08/96	Mr J.J.E. Liebenberg	Wastech Bloemfontein	051-4488230							Done work on pesticides in water	?	96/11/11	
29/08/96	Prof. Oelofsen	University of Port Elizabeth	041-5042111				Prof. G. Batle	UPE Botany	041-5042396		No		
29/08/96	Mr O. Spanenberg	Spanenberg Laboratories	05373-22690				Prof. Hodgson	Inst. Ground Water	051-4019111		No		
28/08/96	Mr L. Bredendam	Dept. Water & Forestry	012-2992482	012-3270321	Instances not stated						Yes	26/09/96	
28/08/96	Prof. Hodgson	Inst. Ground & Water studies	051-4019111	051-4473541							No		
28/08/96	Mr Tharion	Waterlab Pretoria	012-3491044	012-3491072						Will send information if substances are found	Yes	20/09/96	
28/08/96	Prof. G. Batle	University of Port Elizabeth Botany	041-5042396				Dr C. Archibald	CSIR Durban			No		
30/08/96	Dr C. Archibald	CSIR Wastech	031-2618161	031-812509							No		
96/02/09	Mr M. Naude	Mthathuze Water	0351-974213				Mr W. Wepster	University of Zululand Zoology	0351-93911		No		
96/02/09	Mr P. Grobler	Phalaborwa Water	01524-86823								No		
30/08/96	Mrs N. Basson	Goldfield Water	0565-4361	0565-2471	Mrs L. Sanderson	Goldfield Water					Yes	Received fax	
30/08/96	Mr E. Smut	Northern Water	0251-38121								No		
30/08/96	Mr E.E. Ball	Albany Coast Water Board	0464-81231							Expect results in mg/l	No		
30/08/96	Mr Tharion	Overberg Water	02934-22028		Mr D. Vlijm	Overberg Water	Mr M. Louw	CSIR Cape Town	021-8875101		No		
30/08/96	Mr Maritz	Kalahari East Water Board	054-25481				District of Benode Orange River		054-22197		No	19/09/96	
96/04/11	Mr R. Palmer	Karoo Geelkoppes Water Board	054-25605				Mrs B. Conradie	Water Affairs Uppington	054-25605		No		
96/02/09	Mr Grobler	Bosveld Waterboard	01524-86823				Mr D. Rouse				No		
30/08/96	Mrs I. Botha	Van der Byl Park Laboratory	016-333333								No		
30/08/96	Dr van Vuuren	Vandoc	016-549821				Mr C. Jacobs	Municipality Vereeniging	016-503066	Done analysis on vitamins B's in water	No	96/05/08	
30/08/96	Mr A. van Niekerk	Benode Orange District	054-22197	054-23132	Mr W.L. Reichert						Yes	96/06/09	Received fax & literature
30/08/96	Mrs B. Conradie	Dept. Water Affairs Uppington	054-25605	054-25609			Mr B. Benade	Kimberley Nature Conservation			No		
96/02/09	Mr C. Jacobs	Municipality Vereeniging	016-503911								No		
30/08/96	Mrs O. Wind	Strata Laboratory	018-4622089					West Transvaal Water Works	018-4622089		No		
30/08/96	Mr M. Louw	CSIR Cape Town	021-8875101				Mr McConkey	Department of Water Affairs			No		
							Dr P. van Kooten		012-4209215		No		
							Mr D. Whyte		012-8412261		No		
							Mr H. van Duijn				No		
30/08/96	Anonymous	Municipality Nelspruit	013-7599111				BM Kirk Laboratories	Pretoria			No		
30/08/96	Mr K. Auerwald	Laherve Nelspruit	013-7412551				Mrs E. Elwood	ITBC	013-7532071		No		
96/02/09	Mr G. McConkey	Dept. Water & Forestry Cape Town	021-2507100								No		
96/02/09	Dr P. van Kooten	Retired citizen	012-437045	012-8414653							No	19/09/96	Received fax 7/10/96

Institutions contacted for tracing of estrogen and estrogen-mimics in the South African water environment

Date	Contact person	Organization	Tel. no.	Fax no.	Referred to:	Organization	Reference(s)	Ref. organization	Ref. tel no.	Remarks	Response	Contact updated	Response of update call
96/02/99	Mr D. Whyte	Environmentek	012-8412261				Mr O. Jema		012-8414638		No		
96/02/99	Mr G. Jema	Environmentek	012-8414638				Miss L. Slabbert		012-8413953		No		
96/04/99	Dr H. Bowman		012-8080952				Dr W. van Niekerk	AEK / Infotek	012-3162765		No		
96/02/99	Mrs E. Eilwood	Institute of Tropical & Subtropical studies	013-7532071	013-7521854	Mrs A. Thonissen		Mr H. van Wyk	University of Stellenbosch	021-8083222		No	30/09/96	
96/02/99	Miss L. Slabbert	Environmentek	012-8413953	012-8414785			Mrs Louhser	Forensic Dept. Cape Town			No	19/09/96	
96/04/99	Dr W. van Niekerk	Atom Energy Commission (AEC) & Infotek	012-3162765	012-3162945						Does work on the bioassays of these substances	No		
96/02/99	Mr C. Brand	Rural Waterworks	012-3451185							Scheduled a visit - 4 November 1996	Yes		
96/04/99	Mr T. Bailey	Municipality Durban	031-3024793	031-3024747						Pipes lined with phenols - later they chip off	No		
96/03/99	Mr V. Wepener	University of Zululand - Zoology	8229	031-971142						Does testing for these substances	No	19/09/96	
96/03/99	Prof. Tereblanche	University of Zululand - Biochemistry	8229	031-971735						Does work on PAHs - will send results	No	27/09/96	
96/03/99	Dr J. Day	University of Cape Town - Freshwater Institute	8214	021-6503301	Dr L. London						No	19/09/96	
96/04/99	Dr. Elliot	University of Natal	8216				Mr M. Laing	University of Natal - Microbiology			No		
96/03/99	Dr D. Hughes	Rhodes University - Hydrology	8177				Dr P. Rose	Lari Research Institute	0461-27310		No		
96/03/99	Mr W. Malan	University Stellenbosch	8223				Prof. Engelbrecht	Department of Chemistry	021-8083358		No		
96/03/99	Dr Goldring	University of Witwatersrand - Biochemistry	8228				Prof. Mäkelä	Department of Chemistry	011-7162072		No		
							Prof. Hamman	Medical Biochemistry	011-6472264		No		
96/03/99	Prof. Hamman	University of Witwatersrand - Medical Biochemistry	011-6472264				Dr Eowe	SADGR	011-4899500		No		
96/03/99	Prof. van Tonder	University of the Orange Free State - Geohydrology	8231				Mrs L. Craywagen		051-4012317		No		
96/03/99	Prof. Lupoower	University of the Orange Free State - Biochemistry	8231				Prof. Müller	VOVS - Phamovs			No		
96/04/99	Mr D. Bird	Tedmon Durban	031-2042111	031-222671	Dr Spark	Dept. Chemical Sciences		VOVS - Phamovs	051-4013090		Yes	19/09/96	Received fax 11/10/96
96/03/99	Prof. Orinshel	University of Pretoria - Engineering	012-4203215				Prof. W.A. Pretorius	Water Utilisation	012-4203565		No		
96/03/99	Prof. W.A. Pretorius	University of Pretoria - Water Utilisation	012-4203566				Dr C. Viljoen	Rand Water Board			No		
96/03/99	Prof. Dunkin	Rhodes University - Biochemistry	8177				Dr Kfir	CSIR - Analytical Division	012-8412911		No		
96/04/99	Prof. J.H. Hoffmeyer	University Stellenbosch - Biochemistry	021-8083039				Prof. P. Swart	Watertech - CSIR			No		
96/03/99	Prof. Klomp	University of Cape Town - Biochemistry	8214				Dr Maeder	Biochemistry department			No		
							Dr B. Harding	Biochemistry			No		
								Scientific Services - Cape Town	021-6779094		No		
30/09/96	Prof. P. Rose	Lari Research Institute	0461-27310								No		
96/03/99	Prof. H. Hunk	University of the Orange Free State - Phamovs	051-4013149								No		
96/03/99	Prof. Müller	University of the Orange Free State - Phamovs									No		
96/04/99	Dr Kfir	CSIR - Pretoria	012-8412911				Miss L. Slabbert	CSIR - Pta			No		
96/03/99	Mrs E. Jackson	Municipality Durban	031-3024795	031-3024747	Mr Niker	Durban Municipality					Yes		Received fax
96/03/99	Mr F. Venier	CSIR - Pretoria	012-8413951								No		
96/03/99	Prof. W. Grabow	University of Pretoria - Virology	012-3192351	012-3255550	Prof. Vernaak	University Pretoria - Chem. Pathol					No	19/09/96	
96/03/99	Dr J. Howard	Ungeni Water	0331-411111		Mr M. Summerton	Ungeni Waterboard					Yes		Will send information
96/03/99	Mr M. Laing	University of Natal - Microbiology	0331-2605524	0331-2605919		Waste Technology					No	96/07/10	
96/03/99	Dr P. Ashton	CSIR - Pretoria	012-8412911				Mr F. Venier	CSIR - Pretoria	012-8413951		No		
							Miss L. Slabbert	CSIR		Wants copy of MSc thesis			
							Mr J. Howard	Ungeni Water					
							Prof. Grabow	UP - Virology					
							Dr F. Ashton	CSIR - Pta			No		
96/05/99	Prof. Hamburg	Potchefstroomse Universiteit vir CHO - Zoology	0148-2992385								No		
96/05/99	Mr D. Whyte	CSIR - Watertech	012-8412261	012-8414653						Tests for phenol compounds in water	Yes	19/09/96	Received fax
96/05/99	Dr H. Bowman	Agricultural Research Council	012-8080952				Prof. Hamburg	FU vir CHO			No		Received fax - 5/09/96
							Dr J.H. van Wyk	University of Stellenbosch	021-8083222		No		Received fax - 5/09/96
96/05/99	Dr J.H. van Wyk	University of Stellenbosch - Zoology	021-8083222	021-8082405						Pursuing studies for 1997 at Florida, USA	No		
96/04/99	Mr P. van Zyl	SABS - Residue Department	012-4286446	012-3441568							Yes		Telephone response - 5/09/96
96/04/99	Dr P. Kemperter	Institute for Water Quality Studies	012-8080374	012-8080378							Yes		E-mail response - 5/09/96
96/03/96	Mr C. Schoeman	Cybra Laboratories	011-7287373	011-7285444							Yes		Telephone response - 6/08/96
17/09/96	Dr D.J. Steyn	Rand Afrikaans University - Zoology	011-4892286	011-4892286			Mr D. Roux	CSIR - Watertech	012-8412695	Does work on the fertility of fish	No		
							Mr D. Grobler	Institute for Water Quality Studies	012-8080374		Yes		Received articles
18/09/96	Mr D. Roux	CSIR - Watertech	012-8412695		Mrs H. Badenhorst	Institute for Water Quality Studies				Has methods of detecting substances	Yes		Received e-mail - 15/10/96
18/09/96	Mr D. Grobler	Institute for Water Quality Studies	012-8080374	012-8080378	INOC					Scheduled meeting - 4 November 1996	Yes		
19/09/96	Prof. Vernaak	University of Pretoria - Chemical Pathology	012-3192114		Prof. Ibhuk	University of Pretoria					No	19/09/96	
26/09/96	Dr L. London	University of Cape Town - Community Health	021-4066911	021-4066163						Pesticide detection in water study - 1997	No	27/09/96	Received e-mail
18/09/96	Mrs A. van der Merwe	University of Pretoria - Chemical pathology	012-3192116	012-3289500							No	96/07/10	Received fax
20/09/96	Mr T. Hassett	University of Pretoria - Chemistry	8220							Used LLE in article (July '87)	No		See article for detection tips
20/09/96	Dr R. van Rensburg	CSIR - Envrontech	012-8412911							Worked on vitamin B's in water	No		
23/10/96	Mr M. Keet	Dept. Water Affairs - Pretoria	012-6721111	012-6722885							Yes	96/04/11	
21/10/96	Dr Walwyn	Dept. of Trade & Industries	012-3109820	012-3223459							Yes		Received fax - 29/10/96
21/10/96	Mr Heyns	Dept. of Customs & Excise - Pta	012-3149911	012-3257992						Gave tariff numbers	Yes	23/10/96	
21/10/96	Mrs J.M. de Klerk	Dept. of Customs & Excise - Jhb	011-8733341	011-8746526							Yes		
96/12/11	Mr R. Heath	Rand Water - Vereeniging	016-4214020							Contacted on database	Yes		
96/12/11	Dr S. Mitchell	Water Research Commission	012-3303840		Dr S. Mitchell	Water Research Commission	Mr P. Wsagener	Bloemfontein Municipality		OK use of database	Yes		
96/12/11	Mr M. Claassen	CSIR - Pta	012-8412385							Contact person to see for database	Yes		
96/12/11	Mr P. Waagser	Bloemfontein Municipality	051-4058911							Karp fish are all females at Bloemfontein works	Yes		

Appendix 5 An example of the fax sent to various institutions



Potchefstroomse Universiteit vir Christelike Hoër Onderwys

Potchefstroom University
for Christian Higher Education

RESEARCH INSTITUTE FOR INDUSTRIAL PHARMACY

Private Bag X 6001
Potchefstroom
2520
Tel 299-2268

Liesl van der Merwe Tel: 0148-2992294/6
Fax: 0148-2992291
E-mail: iiflvdm@puknet.puk.ac.za

Dear

Re: telephone conversation regarding estrogen mimicking substances in water

As discussed on the telephone, I am enclosing some information regarding my M.Sc. study, and the motivation behind it.

Recently, some chemicals, present in low doses in water, have been accused of causing genetic defects in the animal and human population. The mode of action is termed estrogen-mimicking because of the estrogen-like effects produced by these chemicals.

The aim of the study is to do a literature survey to identify the substances which might exhibit estrogen-mimicking effects and to identify those substances which are most likely to occur in South Africa. A list of compounds have been made, based on the economical and toxicological importance of these substances.

Methods will be developed for the quantification and qualification of these substances, in extremely low concentrations in water. Water samples (clean and effluent) will be collected from various sources and analysed for the presence of these substances.

The result of this study will indicate the present state of water recovery and purification schemes in the country, and may help to formulate future water treatment policies and set new effluent standards.

If seen in this light, your help regarding this matter, in providing me with information such as the type of substances found in water and the amounts detected, will be greatly appreciated and highly thought of.

Thank you for your time and attention regarding this matter.

Your sincerely
Liesl van der Merwe

ESTROGEN MIMICKING SUBSTANCES THAT ARE LIKELY TO OCCUR IN THE SOUTH AFRICAN WATER ENVIRONMENT

• HERBICIDES

1. Atrazine: 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5 triazine 2,4-diamine
2. Methoxychlor (herbicide): 1,1'-(2,2,2-trichloroethylidene)-bis[4-methoxybenzene]
3. Methylphenol (found in herbicide products / phenolic resins)

• PESTICIDES / INSECTICIDES

1. Chlordecone (commercial name: kepone, CD): decachloro-octahydro-1,3,4-metheno-2H-cyclobuta-pentalen-2-one.
2. DDT: 1,1'-(2,2,2-trichloroethylidene)bis[4-chloro-benzene] OR dichlorodiphenyltrichloroethane
3. Endosulfan: 6,9-methano-2,4,3-benzodioxathiepin,6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro endosulfan -3-oxide OR 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide OR 1,4,5,6,7,7-hexachloro-5-norbornene-2,3-dimethanol cyclic sulfite.
4. Heptachlor (insecticide for control of cotton boll weevil: 1H-1,4,5,6,7,8,8-Heptachloro-3a,4-7,7a-tetrahydro-4,7-methanoindene:
5. Malathion (insecticide): [(dimethoxyphosphinothiol)thio]butanedioic acid, diethyl ester OR mercaptosuccinic acid diethyl ester, s-ester with o,o-dimethyl phosphorothioate. *Sanachem*
6. Mirex: (insecticide, fire retardant for plastics, rubber, paint, paper, and electrical goods) 1,1a,2,2,3,3a,4,5,5a,5b,6-dodecachlorooctahydro-1,3,4-methano-1H-cyclobuta[cd]pentalene.
7. o'p'-DDT: 1-chloro-2-(2,2,2-trichloro-1-(4-chlorophenyl)ethyl)benzene OR 2(o-chlorophenyl)-2-(p-chlorophenyl)-1,1,1,1-trichloroethane
8. p'p'-DDT: 1,1'-(2,2,2-trichloroethylidene)bis[4-chloro-benzene] OR 2,2-bis-(p-chlorophenyl)-1,1,1,1-trichloroethane

• ORGANOCHLORINATED PESTICIDES

1. Aldrin (pesticide): (1 α ,4 α ,4a α ,5 α ,8 α ,8a α)-1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro 1,4,5,8-dimethanophtalene. OR 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro -1,4:5,8 -dimethanonaphtalene (HHDN)
2. Benzophenone: (manufacture of insecticides and anti histamines; fixture in strong perfumes (soaps and shampoos))
3. Butylated hydroxytoluene (BHT): (Anti oxidant in food packaging, PVC, cellulose and certain elastomers, and insect repellents)
4. Chlordane (pesticide): 4,7, methano-1-H-indene -1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-chlordane
5. Dieldrin (pesticide): (1a α ,2 β ,2a α , 3 β ,6 β ,6a α ,7 β , 7a α)-3,4,5,6,9,9-hexachloro-1a,2,2a,3,6,6a,7,7a-octahydro-2,7,3,6 dimethanophtl[2,3b]oxirene OR 1,2,3,4,10,10 hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo OR exo-1,4:5,8 dimethanophtalane.
6. Lindane: (1 α ,2 α ,3 β ,4 α ,5 α ,6 β)-1,2,3,4,5,6-hexachloro-cyclohexane (γ HCH / BHC) (broad spectrum insecticide used in a variety of agricultural, medical and veterinary products. In the USA it is a active component of a shampoo (Kwell) used for the treatment of pediculosis as well as for limited use on food crops.)
7. Toxaphene (organochlorine pesticide)

- **PCB's**

1. Arochlor 1254 : see PCB 1254
2. PCB's (organochlorine compounds eg. dichlorodiphenyltrichloorethane and products of combustion eg. petrol engine) Eg. 2'4'6'trichlor-4-bi-phenylol and 2'3'4'5'tetrachloro-4-biphenylol.

- **PLASTICISERS**

1. BBP (plasticiser, especially in the production of vinyl floors) : 1,2-benzenedicarboxylic acid, and it's butyl and- phenylmethyl-esters
2. Bisphenol A (fungicide in polycarbonates) : 4,4'-(1-methylethylidene)bisphenol
3. DEHA : Bis-(2-ethyl-hexyl)adipate : (manufacture of plastics)
4. p-tert-Butylbenzoic acid (plastic industry; corrosion inhibitor in polystyrene manufacture and in dying)
5. Phtalates : eg. n-butyl phtalate, diphenylphtalate, and ethylhexylphtalate

- **INDUSTRIAL**

1. 4-nitrotoluene (manufacture of dyes)
2. n-Butylbenzene : (petrochemical origin)
3. p-cresol (4-methylphenol) : to produce anti oxidant, UV stabilizer
4. Toluene (industrial solvent)
5. Paper Mill wastes: eg. chlorine bleaches

- **PHENOLS**

1. Alkyl phenolic compounds :
 - 4-octylphenol
 - 4-nonylphenol
 - 4-nonylphenol-di-ethoxylate
 - 4-nonylphenoxycarboxylic acid
2. Alkylphenolic ethoxylates (APE)
3. Chlorophenols

- **PHARMACEUTICALS**

Only a few pharmaceutical raw materials are manufactured locally. None of which are on our list of mimics. Due to the high cost of raw materials, and strict control, major industrial spillage is unlikely. The only contamination that might occur is that caused by contraceptive hormones in sewage systems.

• **MISCELLANEOUS**

1. DDE (a metabolite of DDT, a contaminant in dicofol, a insecticide sprayed on food crops) : 1,1'-(dichloroethenylidene)bis [4-chloro-benzene]
2. Dicofol (acaricide) : 4-chloro- α -(4-chlorophenyl)- α -(trichloromethyl)-benzene-methanol
3. PAH (polycyclic aromatic hydrocarbons)(inhibitor of dihydrofolatereductase) : N(4-aminobenzoyl)-glutamic acid
4. PDDF's : dibenzofurans
5. Tetrahydro-isoquinolines (TIQ) : (research tool in neurobiology) Tetrahydropaperoline : [1-(3'4' dihydroxybenzyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline hydrobromide OR 1-[(3,4-dihydroxyphenyl)methyl]-1,2,3,4-tetrahydro-6,7-isoquinolinediol OR 1-(3,4-dihydroxybenzyl)-1,2,3,4-tetrahydro-6,7-isoquinolinediol.

Appendix 6 Import quantities of estrogen- mimics

Import quantities of estrogen and estrogen mimics.

Substance	Code	Unit	IMPORTS : Dec 1995		IMPORTS : Jan-Dec 1995		IMPORTS : Jan-Dec 1994	
			Quantity	Rand	Quantity	Rand	Quantity	Rand
Heptachlor	2903.59.90	kg	NI	NI	NI	NI	NI	NI
Aldrin	2903.59.90	kg	NI	NI	NI	NI	NI	NI
Chlordane	2903.59.30	kg	NI	NI	107121	3915171	142828	4994974
Methoxychlor	2909.3	kg	4104	455308	50408	4180811	NI	NI
Dieldrin	2910.9	kg	2464	104355	63653	1069434	68515	857002
Benzophenone	2914.39	kg	NI	NI	NI	NI	NI	NI
Endosulfan	2920.90.00	kg	125838	1170997	1720793	16709279	NI	NI
Butylated hydroxytoluene	2930	kg	NI	11331643		121528616	NI	116058008
Malathion	2930.90.90	kg	304024	9220828	2764229	80767144	2717895	78881751
Atrazine	2933.69.30	kg	NI	NI	370260	6595814	841400	12130619
Lindane	2903.51	kg						
Methylphenol	Could not classify							
Chlordecone	Could not classify							
DDT	Could not classify							
Mirex	Could not classify							
op' DDT	Could not classify							
pp' DDT	Could not classify							
Toxaphene	Could not classify							
NI = not indicated in tables received								
The import values of the rest of the estrogen and estrogen-mimics were regrettably not received from the Department of Customs and Excise								

Appendix 7 Directory of industrial utilisation of estrogen and estrogen-mimics in South Africa

Directory of industrial utilisation of estrogen and estrogen-mimics in South Africa *.

Substance	Utilisation in the SA situation				
Atrazine	Helm SA 4	<i>Codes:</i> 1 = Local manufacture 2 = Local acquisition, blending, repacking 3 = Imported (ex stock) 4 = Imported (indent basis) 5 = No information provided LCS = Laboratory Chemical Supplier			
	Sanachem 1				
	Transworld group 2				
	Wefco Marketing 2,4				
Chlordane	Wefco Marketing 3,4				
2,4 D	Afro Global Group 4				
	Helm SA 1				
	Sanachem 1				
	Wefco Marketing 2,4				
Estrone	Roussel Laboratories 4				
Endosulfan	Helm SA 4				
	Sanachem 4				
	Wefco Marketing 3,4				
Heptachlor	Wefco Marketing 3,4				
Benzophenone	Akulu - Marchon 3				
	BASF SA 3,4				
	Gunther 3				
	Merck (LCS) 4				
	Sharon Bolel Chem Mark 3				

Directory of industrial utilisation of estrogen and estrogen-mimics in South Africa *.

Substance	Utilisation in the SA situation				
Butylated Hydroxytoluene	Betpharma 3,4				
	CJ Petrow 2	Codes: <i>1 = Local manufacture</i> <i>2 = Local acquisition, blending, repacking</i> <i>3 = Imported (ex stock)</i> <i>4 = Imported (indent basis)</i> <i>5 = No information provided</i> <i>LCS = Laboratory Chemical Supplier</i>			
	Crest Chemicals 3,4				
	Helm SA 4				
	Hochfeld Fine Chemicals 3,4				
	Holpro Fine Chemicals 1				
	Nisa Chemicals 3				
	Orchem 3				
	Protea Industrial Chemicals 3				
	Savannah Fine Chemicals 3				
Lindane (β - BHC)	Afro Global Group 4				
	Wefco Marketing 3,4				
Bisphenol A	First Chemicals 4				
	Helm SA 4				
	Rhône - Poulenc Chemicals 4				
	Shell Chemicals 4				
p - tert - Butyl benzoic acid	Hoechst 4				
	Shell Chemicals 4				
p - Cresol	Sasolchem 1				
Toluene	Associated Chemical Enterp. 3				
	B & R Products 2				
	Betpharma 2				
	CJ Petrow 2				
	Crest Chemicals 2				

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Substance	Utilisation in the SA situation				
Toluene (continued)	Kimcor SA 4				
	Merck (LCS) 3	Codes: <i>1 = Local manufacture</i> <i>2 = Local acquisition, blending, repacking</i> <i>3 = Imported (ex stock)</i> <i>4 = Imported (indent basis)</i> <i>5 = No information provided</i> <i>LCS = Laboratory Chemical Supplier</i>			
	Mikrochem / Ortochem 2,1				
	Nisa Chemicals 2				
	Protea Industrial Chemicals 2,3,4				
	Saarchem - Holpro An (LCS) 2,3				
	SMM Chemicals 2,3,4				
	Suprachem 1				
	Triangle Chemical Ind. 1				
Alkylphenol ethoxylates	Polycor Chemicals 3				
Dicofol	Helm SA 4				
Nonylphenol	CJ Petrow 3				
	First Chemicals 3				
	ICI (SA) 3				
Nonylphenol ethoxylates	Chemical & Indust. Marketing 3				
	Crest Chemicals 3				
	First Chemicals 4				
	ICI (SA) 3				
	Nisa Chemicals 3				
	Polycor Chemicals 3				
	Protea Industrial Chemicals 3				
	Rhône Polenc Chemicals 4				
	Savannah Fine Chemicals 3				
	Union Carbide SA 3				
Octyl phenol	ICI (SA) 3				

Directory of industrial utilisation of estrogen and estrogen-mimics in South Africa *.

Substance	Utilisation in the SA situation				
Chlorophenol	Rhône - Polenc Chemicals 4				
	SMM Chemicals 2,3,4	<i>Codes:</i> 1 = Local manufacture 2 = Local acquisition, blending, repacking 3 = Imported (ex stock) 4 = Imported (indent basis) 5 = No information provided LCS = Laboratory Chemical Supplier			
Levonorgestrel	Not indicated				
Lynoeestrenol	Not indicated				
Ethinylestradiol	CJ Petrow 3				
	Roussel Laboratories 3,4				
Ethisterone	Frankel Chemicals 4				
	Roussel Laboratories 4				
Ethynodiol diacetate	Not indicated				
Estradiol benzoate	Not indicated				
Estradiol phenylpropionate	Not indicated				
Norethisterone	Not indicated				
Norethisterone acetate	Not indicated				
Nortehisterone enantate	Not indicated				
Norgestrel	Not indicated				
Mestranol	Not indicated				

Directory of industrial utilisation of estrogen and estrogen-mimics in South Africa *

Substance	Utilisation in the SA situation	<i>Codes:</i> 1 = Local manufacture 2 = Local acquisition, blending, repackaging 3 = Imported (ex stock) 4 = Imported (indent basis) 5 = No information provided LCS = Laboratory Chemical Supplier			
Medrogestone	Not indicated				
Gestodene	Not indicated				
Gestrone	Not indicated				
Diethylstilboestrol diphosphate	CJ Petrow 3				
	Rogoff Chemicals 3,4				
Oestradiol	Rogoff Chemicals 3,4				
Oestradiol benzoate	Roussel Laboratories 4				
Oestradiol diethanate	Roussel Laboratories 4				
Oestradiol succinate	Not indicated				
Conjugated oestrogens	Frankel Chemicals 4				
EPA (5,8,11,14,17 - eicopentanoic acid)	Roche products 3				
d - Methoxyphenol (guaiacol)	Globe Pharmaceuticals (SA) 3				
	Merk (LCS) 3				
	Rhône Polenc Chemicals 4				
	Sharon Bolcl Chem Mark 3				

Codes:
1 = Local manufacture
2 = Local acquisition, blending, repack
3 = Imported (ex stock)
4 = Imported (indent basis)
5 = No information provided
TCS = Laboratory Chemical Supplier

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Codes:

- 1 = Local manufacture
- 2 = Local acquisition, blending, repackaging
- 3 = Imported (ex stock)
- 4 = Imported (indent basis)
- 5 = No information provided
- 6 = LCS = Laboratory Chemical Supplier

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Substance	Utilisation in the SA situation				
Musk ketone	Gunther 3	Codes: <i>1 = Local manufacture</i> <i>2 = Local acquisition, blending, repacking</i> <i>3 = Imported (ex stock)</i> <i>4 = Imported (indent basis)</i> <i>5 = No information provided</i> <i>LCS = Laboratory Chemical Supplier</i>			
	Sharon Bolel Chem Mark 3				
Musk xylene	Not indicated				
n - Butylbenzene	Not indicated				
Mycotoxins	Not indicated				
Norethisterone	Not indicated				
o' Phenylphenol	Not indicated				
op' DDD	Not indicated				
op' DDE	Not indicated				
Octylphenol	ICI (SA) 3				
op' DDT	Not indicated				
p'p' DDE	Not indicated				
p - Hydroxybenzoic acid	Not indicated				
p'p' DDT	Not indicated				
Polycyclic aromatic hydrocarbons (PAH)	Not indicated				

Substance	Utilisation the SA situation
PCBs : Dichlorodiphenyltrichloroethane	Not indicated
2'4'6'-trichlor-4-di-phenylol	Not indicated
2'3'4'5-tetrachloro-4-biphenylol	Not indicated
Polychloronated dibenzodifurans (PCDD)	Not indicated
Dibenzofurans (PDDF)	Not indicated
Pentachlorophenol	Not indicated
Phenol red	Not indicated
Phloretin	Not indicated
Phytoestrogens : coumestrol	Not indicated
genistein	Not indicated
salsoline	Not indicated
Prednisolone	Frankle Chemicals 4
	Helm SA 4
	Holpro Fine Chemicals 4
	Rogoff Chemicals 3,4
	Roussel Laboratories 3,4
	Sharon Bolel Chem Mark 4
Tetrachloro-dibenzodioxin (TCDD)	Not indicated
Tetrahydro-isouquinolines (TIQ)	Not indicated

Codes:

1 = Local manufacture

2 = Local acquisition, blending, repacking

3 = Imported (ex stock)

4 = Imported (indent basis)

5 = No information provided

ICS = Laboratory Chemical Supplier

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Substance	Utilisation the SA situation	Codes:
Toremifene	Not indicated	1 = Local manufacture
		2 = Local acquisition, blending, repackaging
Toxophene	Not indicated	3 = Imported (ex stock)
		4 = Imported (indent basis)
Zearelenone	Not indicated	5 = No information provided
		LC5 = Laboratory Chemical Supplier

* Compiled from CSIR Informational Services. Chemdata. (1995/1996): 1-129

Appendix 8 Chemical characteristics of estrogen and estrogen-mimics

<i>Classification</i>	<i>Estrogen / estrogen-mimic</i>	<i>Melting points</i>	<i>Water solubility</i>	<i>Medium found in</i>	<i>Bibliographical source</i>
Herbicides	2,4 D	138°C	Almost insoluble in water	Not specified	(Raloff, 1994:56-58)
	Atrazine	171-174°C	Soluble in water at 25°C 70 ppm	Dam water	(Coetzee & Vahrmeijer, 1986:178)
	Dibenzodioxin-2,3,7,8-tetrachlorodioxin	295°C	Not indicated	Not specified	(Rennie, 1993:15)
	Methoxychlor	78-78.2°C	Insoluble in water	Not specified	(Hileman, 1994:19-23 ; Lee Davis & Bradlow, 1995:144-149 & Thomas <i>et al.</i> , 1993:147-151)
	Methylphenol	35.5°C	Slightly soluble in water	Polluted waters	(Geibler & Scholer, 1994:2047-2053)
Pesticides / Insecticides	Chlordecone (Kepone, CD)	Not indicated	Slightly soluble in water	Not specified	(Soto <i>et al.</i> , 1994: 380-384)
	DDT	108.5-109°C	Practically insoluble in water : $1,2 \times 10^{-3} \text{ g/m}^3$ at 20 °C	Not specified	(Raloff, 1994:56-58; Richardson & Bowrin, 1985 & Lee Davis & Bradlow, 1995:144-149)
	Endosulfan	70-100°C	Practically insoluble in water	Not specified	(Begley & Glick, 1994:44; Melius, 1995:77-79; Raloff, 1994:56-58 & Soto <i>et al.</i> , 1994:380-384)
	Heptachlor	95-96°C	Not indicated	Not specified	(Raloff, 1984:24-27 & Raloff, 1993:10-15)
	Kepone	See chlordecone	See chlordecone		
	Malathion	2.9°C	Slightly soluble in water	Not specified	(Dutta <i>et al.</i> , 1994:215-220)
	Mirex	Not indicated	Practically insoluble in water	Not specified	(Hansen & Jansen, 1994:525-527 & Raloff, 1984:24-27)
	o'p' DDT	108.5-109°C	Practically insoluble in water	Not specified	(Forster <i>et al.</i> , 1975:1777-1780 Hileman, 1994:19-23 & Soto <i>et al.</i> , 1994:380-384)
	p'p' DDT	108.5-109°C	Practically insoluble in water	Not specified	(Hileman, 1994:19-23 & Mestel, 1994:9:9 & Bustos <i>et al.</i> , 1988: 396-502)

<i>Classification</i>	<i>Estrogen / estrogen-mimic</i>	<i>Melting points</i>	<i>Water solubility</i>	<i>Medium found in</i>	<i>Bibliographical source</i>
Pesticides / Insecticides	β -Hexachlorocyclohexane (β -HCH)	112.5°C	Insoluble in water	Snow and rain	(Fingler <i>et al.</i> , 1994:1135-1140)
(continued)					
	Endosulfan (alpha isomer)	See endosulfan	See endosulfan	See endosulfan	See endosulfan
	Endosulfan (beta isomer)	See endosulfan	See endosulfan	See endosulfan	See endosulfan
	o,p' DDE	Not indicated	Not indicated	Not indicated	See DDT
	p,p' DDE	Not indicated	Not indicated	Muscle tissue of fish	(Roux <i>et al.</i> , 1994:333-340)
Organochlorinated	Aldrin	104°C	Insoluble in water	Not specified	
Pesticides	Benzophenone	139-140°C	Not indicated	Not specified	
	Butylated hydroxytoluene (BHT)	70°C	Insoluble in water	Not specified	
	Chlordane	Not indicated	Insoluble in water	Not specified	(Scafferner <i>et al.</i> , 1983 ; Soto <i>et al.</i> , 1992:295-309 & Lee Davis & Bradlow, 1995:144-149)
	Dieldrin	176-177°C	Practically insoluble in water : 0.25 g/m ³	Not specified	(Begley & Glick, 1994:44; Raloff, 1994:56-58 & Soto <i>et al.</i> , 1992:295-309)
	Lindane (gamma BHC)	112.5°C	Insoluble in water	Not specified	(Warhurst, 1995b:92-756)
	Toxaphene	65-90°C	Practically insoluble in water	Not specified	(Melius, 1995:77-79; Raloff, 1994:56-58 & Soto <i>et al.</i> , 1994:380-384)
	Arochlor 1254	No open cup to flashpoint	Not indicated	Not specified	(Safe, 1994:87-149)
	Arochlor 1260	No open cup to flashpoint	Not indicated	Not specified	(Ando <i>et al.</i> , 1985:51-57 & Roux <i>et al.</i> , 1994)

	<i>Estrogen / estrogen-mimic</i>	<i>Melting points</i>	<i>Water solability</i>	<i>Medium found in</i>	<i>Bibliographical source</i>
Classification					
Organochlorinated pesticides (continued)	PCB's e.g. organochlorine compounds like:			Fish samples	(Stone, 1992:308-310)
	2'4'6trichlor-4-biphenylol	Not indicated			
	2'3'4'5'tetrachloro-4-biphenylol	Not indicated			
	dichlorophenyltrichloorethane	Not indicated			
	Some PCB's	Not indicated			
Plasticisers	BBP	Not indicated	Not indicated	Not specified	(ANON., 1995D:575)
	Bisphenol A	150-155°C	Practically insoluble in water	Not specified	(Bradfield <i>et al.</i> , 1988:229-237 & Lee Davis & Bradlow, 1995:144-149)
	Bis(2-ethylhexyl)adipate (DEHA)	Not indicated	Not indicated	Not specified	
	p-tert-Butylbenzoic acid	Not indicated	Not indicated	Not specified	
	Phtalates e.g. n-butyl phtalate	Not indicated	Sparingly soluble in water	Not specified	(ANON., 1995D:575; Hansen & Jansen, 1994:525-527 & Warhurst, 1995b:92a:756)
	diphenylphtalate	Not indicated	Not indicated	Not specified	
	ethylhexylphtalate	Not indicated	Not indicated	Not specified	
	Di(2)ethylhexyl phtalates	Not indicated	Not indicated	Not specified	(ANON., 1995D:575)
	Plastics	Individual variation	Individual variation	Not specified	(Lee Davis & Bradlow, 1995:144-149)
	Polycarbonated plastics and waterjugs	Not indicated	Insoluble in water	Not specified	(Begley & Glick, 1994:44)
	Polystyrene tubing	Not indicated	Insoluble in water	Not specified	(Raloff, 1993:10-15)
Industrial materials	3,9 - Dihydroxybenz(a)anthracene	Not indicated	Not indicated	Not specified	(Raloff, 1993:10-15)
	4-Nitrotoluene	Not indicated	Insoluble in water	Not specified	
	n-Butylbenzene	Flash point (open cup): 71°C	Insoluble in water	Not specified	
	p-Cresol	35.5°C	Slightly soluble in water	Not specified	
	Toluene	Minus 95°C	Slightly soluble in water	Not specified	
	Paper mill wastes e.g. chlorine bleaches	Varies	Varies	Not specified	(Raloff, 1995:44-46)
	Corn oil and related poly unsaturated fats	Minus 18-10°C	Insoluble in water	Not specified	(Lee Davis & Bradlow, 1995:144-149)
	Fuel constituents : aromatic hydrocarbons	Individual variation	Individual variation	Not specified	(Lee Davis & Bradlow, 1995:144-149)

<i>Classification</i>	<i>Estrogen / estrogen mimic</i>	<i>Melting points</i>	<i>Water solubility</i>	<i>Medium found in</i>	<i>Bibliographical source</i>
Industrial materials (continues)	Phenol red	Not indicated	Not indicated	Not specified	(Ernst <i>et al.</i> , 1989:907-914)
Phenols	2,2'dihydroxy 5' dichlorophenylfenol	Not indicated	Not indicated	Not specified	(Wood & Richardson, 1978:491-496)
	Alkylphenolic compounds:			Not specified	(Ginsburg, 1994:284-285:284-285; Raloff, 1984:24-27; & Mestel, 1994:9:9)
	e.g. 4-octylphenol	Not indicated	Not indicated		
	4-nonylphenol	Flash point (open cup): 300°F	Practically insoluble in water		
	4-nonylphenol-di-ethoxylates	Not indicated	Not indicated		
	4-nonylphenoxycarboxylic acid	Not indicated	Not indicated		
	Alkylphenolic ethoxylates (APE's)	Not indicated	Not indicated	Treated drinking water Sewage sludge	(ANON., 1993b:11) (Purdom <i>et al.</i> , 1994:275-285 & Scafferner <i>et al.</i> , 1983)
	Alkylphenolic poly ethoxylates (APEO's)			Not specified	(ANON., 1995D:575; Ginsburg, 1994:284-285 & White <i>et al.</i> , 1994:175-182)
	e.g. nonylphenolpolyethoxylates	Not indicated	Not indicated		
	octylphenolpolyethoxylates	Not indicated	Not indicated		
	APEC	Not indicated	Not indicated	See APE's	See APE's
	Dichlorophenol	64.5-65.5°C	Not indicated	Not specified	
	Dimethylphenol	Not indicated	Not indicated	Not specified	
	Pentachlorofenol	190-191°C	Almost insoluble in water	Not specified	(Wood & Richardson, 1978:491-496)
	Alkyl phenols : pentaphenol to nonylphenol	Individual variations	Individual variations	Not specified	(Hileman, 1994:19-23 & Warhurst, 1995b:92a:756)
	1,1, Dimethylethyl-4-methoxy-phenol (BHA)	48-55°C	Insoluble in water	Not specified	(ANON., 1995D:575)
	Nonylphenol	Flash point (open cup): 300°F	Practically insoluble in water	Sewage sludge	(Purdom <i>et al.</i> , 1994:275-285)
	o' Phenylphenol	164-165°C	Not indicated	Not specified	(Bicknell <i>et al.</i> , 1995:7-9 & Wood & Richardson, 1978:491-496)
	Octylphenol	Not indicated	Not indicated	Not specified	(Bardin <i>et al.</i> , 1988:933-974)

<i>Classification</i>	<i>Estrogen / estrogen mimic</i>	<i>Melting points</i>	<i>Water solubility</i>	<i>Medium found in</i>	<i>Bibliographical source</i>
Plant substances containing	Anthocyanidins	Not indicated	Not indicated	Natural plant estrogens	(Miksicek, 1993:37-43)
estrogen-like compounds	Chalones	Not indicated	Not indicated	Natural plant estrogens	(Miksicek, 1993:37-43)
	Coumestrol	385°C	Insoluble in water at acidic/neutral pH	Natural plant estrogens	(Miksicek, 1993:37-43; Shore <i>et al.</i> , 1992:271-222 & Whitten <i>et al.</i> , 1995:771s-776s)
			Sparingly soluble in water at alkaline		
	Enterolactone	141-143°C	Not indicated	Not specified	(Welshons <i>et al.</i> , 1987:169-175)
	Equol	Not indicated	Not indicated	Not specified	(Welshons <i>et al.</i> , 1987:169-175)
	Flavonols	Not indicated	Not indicated	Natural plant estrogens	(Miksicek, 1993:37-43)
	Flavones	99-100°C	Insoluble in water	Natural plant estrogens	(Miksicek, 1993:37-43)
	Flavonones	Not indicated	Not indicated	Natural plant estrogens	(Miksicek, 1993:37-43)
	Isoflavones	Individual variation	Individual variation	Natural plant estrogens	(Miksicek, 1993:37-43)
	Phloretin	Not indicated	Insoluble in water	Natural plant estrogens	(Miksicek, 1993:37-43)
	Phytoestrogens e.g. coumestrol	See coumestrol	See coumestrol	Not specified	(Larkin, 1995:144-149)
	genistein	297-298°C	Insoluble in water		
	salsolidine	Not indicated	Not indicated		
	salsoline	221°C	Slightly soluble in water		
	salsolinol	Not indicated	Not indicated		
	Ricinus communaris var. minor (seed)	Not indicated	Not indicated	Not specified	
	Soyabeans	Not indicated	Not indicated	Not specified	(Vines, 1993:5:5)
	Soy	See soybeans	See soybeans	Not specified	(Ginsburg, 1994:284-285)
	Zearelenone	164-165°C	Insoluble in water	Contains mycotoxins	(Shoental, 1985:15)
	Alliquotes of the extracts tested for total estrogen				
	content of the Medicago Sativa plant (alfalfa plant)	Not indicated	Not indicated	Not specified	(Shore <i>et al.</i> , 1992)

<i>Classification</i>	<i>Estrogen / estrogen mimic</i>	<i>Melting points</i>	<i>Water solubility</i>	<i>Medium found in</i>	<i>Bibliographical source</i>
Pharmaceutical products	Adiol	173-174°C	Not indicated	Not specified	(Leroy <i>et al.</i> , 1988: 243)
	Caffeine	238°C	Soluble in water	Not specified	
	Cimetidine	141-143°C	Soluble in water	Not specified	(Lee Davis & Bradlow, 1995:144-149)
	Clenbuterol	174-175.5°	Very soluble in water	Not specified	(Re <i>et al.</i> , 1993:438-442)
	Cyclohexymide	119-121°C	Slightly soluble in water	Not specified	(Perez <i>et al.</i> , 1993:233-243)
	Ginseng	Not indicated	Not indicated	Found in face cream	(Hopkins <i>et al.</i> , 1988:1121-1122)
	Mifepristone (RU 486)	150°	Not indicated	Not specified	(Dibbs <i>et al.</i> , 1995:134-140)
	o'p' DDD	76-78°C	Not indicated	Not specified	(ANON., 1993b:11)
	p-Hydroxy benzoic acid	213-214°C	Slightly soluble in water	Not specified	
	Synthetic estrogens e.g. Diethylstilboestrol	169-172°C	Almost insoluble in water	Drinking water	(Aherne & Briggs, 1989:735-136 & Rurainski <i>et al.</i> , 1977:288-291)
	Ethinylestradiol Mestranol Estradiol benzoate	141-146°C 148-154°C 191-196°C	Practically insoluble in water	Sewage effluent, riverwater, potable water, & drinking water	(Aherne & Briggs, 1989:735-136; ANON. 1993b:11 & Rathner & Sonneborn, 1979:45-49)
	Prednisolone	Not indicated	Very slightly soluble in water	Not specified	(Mahli <i>et al.</i> , 1991:690-693)
	EPA (5,8,11,14,17 - Eicopentaenoic acid)	Not indicated	Oil - thus insoluble in water	Not indicated	(Budavari <i>et al.</i> , 1989: 3499)
Naturally occurring substances	Boron	2200°C	Not indicated	Not specified	(Nielsen <i>et al.</i> , 1992:237-246)
	Broccoli	Not applicable	Not applicable	Not specified	(ANON., 1994B:414-416)
	Cholesterol	148.5°C (anhydrous)	Insoluble in water	Not specified	(Moore <i>et al.</i> , 1991:85-97)
	Immunoglobulin G (IgG)	Not indicated	Not indicated	Not specified	(Borkowski <i>et al.</i> , 1991:3238-3292)
	Milk from pregnant cows	Minus 0.54°C	soluble in water	Milk of cows	(Vines, 1993:5)
	Mycotoxins	Not indicated	Not indicated	From <i>Fusarium</i> moulds	(Scheontal, 1985:15)
	Phytohormones	Individual	Individual variation	Not specified	(Soto <i>et al.</i> , 1992:295-309)
	Resorcylic acid lactones	213°C	soluble in hot water	Not specified	(Wilcox & Wahlquist, 1990:905-906)
	Xenobiotics	Not indicated	Not indicated	Not specified	(Palmer & Palmer, 1995:19-25 & Thomas <i>et al.</i> , 1993:147-151)
	Xenoestrogens (foreign estrogens)	Individual variation	Individual variation	Not specified	(Lee Davis & Bradlow, 1995:144-149)

	<i>Estrogen / estrogen mimic</i>	<i>Melting points</i>	<i>Water solubility</i>	<i>Medium found in</i>	<i>Bibliographical source</i>
<i>Classification</i>					
Endogenous estrogens	Enterodiol	Not indicated	Not indicated	Not specified	(Shore <i>et al.</i> , 1993:361-366)
	Estrone	251-254°C	Almost insoluble in water	Not specified	(Shore <i>et al.</i> , 1993:361-366)
	Estradiol	173-179°C	Almost insoluble in water	Drinking water	(Meikle <i>et al.</i> , 1994; Mor <i>et al.</i> , 1992:3633-3640; Mueller & Kim, 1978:1429-1431 & Rurainski <i>et al.</i> , 1977:288-291)
Miscellaneous	1-Hydroxychlordene	Not indicated	Not indicated	Not specified	(ANON., 1993b:11)
	Benzo(a)pyrene	179-179.3°C	Practically insoluble in water	Not specified	(Weiss, 1994:169-175)
	DDE	See DDT	See DDT	Not specified	(Raloff, 1984:24-27; Raloff, 1995:44-46; Taubes, 1994:499-500 & Weiss, 1994:169-175)
	Dicofol	77-78°C	Insoluble in water	Not specified	(Raloff, 1984:24-27)
	Poly aromatic hydrocarbons (PAH's)	Individual	Individual variation	Not specified	(Raloff, 1993:10-15)
	Tetrahydro-isoquinolines (TIQ)	Individual	Individual variation	Not specified	(Stammel <i>et al.</i> , 1991:1319-1330)
	PDDF's	Individual variation	Individual variation	Not specified	(Fifield, 1995:44-54)
	Estrogens found in sewage water	Not indicated	Individual variation	Lake & drinking water	(ANON. 1993 & Shore <i>et al.</i> , 1993:361-366)
	Endrin	Not indicated	Not indicated	Not specified	
	3 Adihidroxybenz(a)antracene	Not indicated	Not indicated	Not specified	
	Aromatic hydrocarbons	Not indicated		Not specified	(Giesy <i>et al.</i> , 1994:128-135 & Poland & Knutson, 1982:517-554)
	Carboxylic acid	Individual variation		Not specified	(White <i>et al.</i> , 1994:175-182)
	Chicken manure	Not applicable	Insoluble in water	From the chicken	(Shore <i>et al.</i> , 1993:361-366)
	Chlorinated hydrocarbons	Individual	Individual variation	Not specified	(Dibb, 1995:27-31)
	Chlorinated organic compounds e.g. atrazine		See atrazine	See atrazine	See atrazine
	chlordane		See chlordane	See chlordane	See chlordane
	DDT		See DDT	See DDT	See DDT
	endosulfan		See endosulfan	See endosulfan	See endosulfan
	kepone		See kepone	See kepone	See kepone
	methoxychlor		See methoxychlor	See methoxychlor	See methoxychlor
	some PCB's		See individual PCB's	See individual PCB's	See individual PCB's

<i>Classification</i>	<i>Estrogen / estrogen mimic</i>	<i>Melting points</i>	<i>Water solubility</i>	<i>Medium found in</i>	<i>Bibliographical source</i>
Miscellaneous (continued)	Chlorine compounds that bleach paper	Individual	Individual variation	Not specified	(Begley & Glick, 1994:44)
	Clone ID5	Not indicated	Not indicated	Not specified	(Mor <i>et al.</i> , 1992:3633-3640)
	Crops grown in animal pastures	Not indicated	Insoluble in water	Crops in pastures	(Jenkins <i>et al.</i> , 1957:278)
	Bis (4-chlorophenyl)-methanone (DBP)	Not indicated	Not indicated	Found in phthalates	(ANON., 1995D:575)
	Bis (ethylhexyl)phtalate (DEHP)	Not indicated	Not indicated	Found in phthalates	(ANON., 1995D:575)
	Dimethylbenz(a)anthracene (DMBA)	Not indicated	Insoluble in water	Not specified	(Pasqualini <i>et al.</i> , 1988:6434-6437)
	Epidermal growth factor (human)	Not indicated	Not indicated	Not specified	(Ignar - Trowbridge <i>et al.</i> , 1992:4658-4662)
	Manure of swine, horses, goats, and cows	Not indicated	Insoluble in water	Derived from the animals	(Shore <i>et al.</i> , 1993:361-366)
	Milk	Minus 0.54°C	Soluble in water	From animals	(Shore <i>et al.</i> , 1993:361366 & Vines, 1993:5:5)
	Musk ketone (scent)	Not indicated	Not indicated	Not specified	
	Musk xylene (scent)	Not indicated	Not indicated	Not specified	
	Organochlorines	Individual	Individual variation	Drinking water	(ANON. 1985:218)
	Poly chlorinated dibenzo-p-dioxins (PCDD)	Individual variation	Individual variation	Not specified	(Dibb, 1995:27-31 & Raloff, 1995:44-46)
	Petroleum by-products	Individual variation	Individual variation	Not specified	(Raloff, 1993:10-15 & Rennie, 1993:15)
	Tetra chloro-dibenzo-dioxins (TCDD)	295°C	Not indicated	Pulp & papermill effluent	(Zacharewski <i>et al.</i> , 1995:2140-2416)
	Tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQ)	Individual variation	Individual variation	Not specified	(Fifield, 1995:44-54)

* Compiled from Budavari *et al.*, (1989:1-app3)

**Appendix 9 A list of the estrogen and estrogen-
mimics to be tested for in the South
African water environment.**

Appendix 9 : A list of estrogen and estrogen-mimics that are t

Classification	Estrogen / estrogen mimic	Water solubility *	Toxicity (LD50 and LC50 values)	Local Manufacture
Herbicides	Dichlorophenoxyacetic acid (2,4 D)	Almost insoluble in water	LD50 (oral, rat) = 375 mg/kg	Helm SA - Johannesburg, Sanachem - Durban
	Atrazine	Soluble in water at 25°C. 70 ppm	LD50 (oral, rat) = 672 mg/kg	Sanachem - Durban
	Dibenzodioxin-2,3,7,8-tetrachlorodioxin	NOT INDICATED	LD50 (oral, rat) = 1220 mg/kg	
	Methoxychlor	Insoluble in water	LD50 (oral, rat) = 5000 mg/kg	Sanachem - Durban
	Methylphenol	Slightly soluble in water	LD50 (oral, rat) = 1454 mg/kg	Not indicated - Sasolburg
Pesticides / Insecticides	Chlordecone (Kepone, CD)	Slightly soluble in water	Not supplied	Sanachem - Durban
	DDT	Practically insoluble in water : 1,2 x 10 ⁻³ g/m ³ at 20°C	Not supplied	Sanachem - Durban
	Endosulfan	Practically insoluble in water	LD50 (oral, rat) = 55 mg/kg	Sanachem - Durban
	Heptachlor	Not soluble in water	LD50 (oral, rat) = 68 mg/kg	
	Kepone	See chlordecone	LD50 (oral, rat) = 91300 ug/kg	
	Malathion	Slightly soluble in water	LD50 (oral, rat) = 725 mg/kg	Sanachem - Durban
	Mirex	Practically insoluble in water	LD50 (oral, rat) = 235 mg/kg	
	o,p' DDT	Practically insoluble in water	Not supplied	Sanachem - Durban
	p,p' DDT	Practically insoluble in water	Not supplied	Sanachem - Durban
	β-Hexachlorocyclohexane (β-HCH)	Insoluble in water	LD50 (oral, rat) = 100 mg/kg	
	Endosulfan (alpha isomer)	See endosulfan	LD50 (oral, rat) = 76 mg/kg	Sanachem - Durban
	Endosulfan (beta isomer)	See endosulfan	LD50 (oral, rat) = 240 mg/kg	Sanachem - Durban
	o,p' DDE	NOT INDICATED	Not supplied	Polifin - Sasol
	p,p' DDE	NOT INDICATED	Not supplied	Polifin - Sasol
Organochlorinated pesticides	Aldrin	Insoluble in water	LD50 (oral, rat) = 39 mg/kg	
	Benzophenone	Insoluble in water	LD50 (oral, mouse) = 2895 mg/kg	
	Butylated hydroxytoluene (BHT)	Insoluble in water	LD50 (oral, rat) = 890 mg/kg	Holpro Fine Chemicals - Johannesburg & F
	Chlordane	Insoluble in water	LD50 (oral, rat) = 283 mg/kg	
	Dieldrin	Practically insoluble in water : 0.25 g/m ³	Not supplied	
	Lindane (gamma BHC)	Insoluble in water	LD50 (oral, rat) = 265 mg/kg	
	Toxaphene	Practically insoluble in water	Not supplied	Sanachem
Polychlorinated biphenyls (PCB's)	Aroclor 1254	NOT INDICATED	LD50 (oral, rat) = 1010 mg/kg	SASTECH - Sasolburg
	Aroclor 1260	NOT INDICATED	LD50 (oral, mouse) = 1900 mg/kg	SASTECH - Sasolburg
	PCB's e.g. organochlorine compounds		Not supplied	SASTECH - Sasolburg
	like: 2,4,6-trichloro-4-biphenylol		Not supplied	SASTECH - Sasolburg
	2,3,4,5-tetrachloro-4-biphenylol		Not supplied	SASTECH - Sasolburg
	dichlorophenyltrichloroethane		Not supplied	SASTECH - Sasolburg
	some PCB's		Not supplied	SASTECH - Sasolburg
Plastics	Butyl benzyl phthalate (BBP)	NOT INDICATED	LD50 (oral, rat) = >2330 mg/kg	
	Bisphenol A	Practically insoluble in water	Not supplied	Safipol - Sasolburg
	Bis(2-ethylhexyl)adipate (DEHA)	NOT INDICATED	Not supplied	Karbochem - Sasolburg
	p-tert-Butylbenzoic acid	Soluble at 25 °C : 3.4 g/l	LD50 (oral, rat) = 2,565 mg/kg	AECI Industrial Chemicals - Modderfontein
	Phthalates e.g. n-butyl phthalate	Springly soluble in water	Not supplied	
	diphenylphthalate	Insoluble in water	Not supplied	
	ethylethylphthalate	NOT INDICATED	LD50 (oral, rat) = 30 gm/kg	
	Plastics	Individual variation	Not supplied	
	Polycarbonated plastics and waterjug	Insoluble in water	Not supplied	
	Polystyrene tubing	Insoluble in water	Not supplied	Safipol - Sasolburg
Industrial materials	Dx2ethylhexyl phthalates	NOT INDICATED	Not supplied	NCP Polymer Materials - Durban
	3,9 - Dihydroxybenz(a)anthracene	NOT INDICATED	LD50 (oral, rat) = 327 mg/kg	
	4-Nitrotoluene	Insoluble in water	Not supplied	
	n-Butylbenzene	Insoluble in water	Not supplied	Not indicated - Sasolburg
	p-Cresol	Slightly soluble in water	LD50 (oral, rat) = 1454 mg/kg	Sasolchem - Durban
	Toluene	Slightly soluble in water	LD50 (subcutaneous, mouse) = 2250 mg/kg	Mikrochem, Orchem, Suprachem, Triangl
	Paper mill wastes e.g. chlorine bleaches	Varies	Not supplied	Sapec - Not indicated
	Corn oil and related poly unsaturated fats	Insoluble in water	Not supplied	
	Fuel constituents : aromatic hydrocarbons	Individual variation	Not supplied	
	Phenol red	Not indicated	Not supplied	
Phenols	2,2'-dihydroxy 5' dichlorophenylphenol	NOT INDICATED	Not supplied	
	Alkylphenolic compounds:		Not supplied	Not indicated - Sasolburg
	eg. 4-octylphenol	NOT INDICATED	Not supplied	
	4-nonylphenol	Practical insoluble in water	Not supplied	Not indicated - Sasolburg
	4-nonylphenol-di-ethoxylates	NOT INDICATED	Not supplied	
	4-nonylphenoxycarboxylic acid	NOT INDICATED	Not supplied	
	Alkylphenolic ethoxylates (APE's)	NOT INDICATED	Not supplied	
	Alkylphenolic poly ethoxylates (APEO's)		Not supplied	
	e.g. nonylphenolpolyethoxylates	NOT INDICATED	Not supplied	
	octylphenolpolyethoxylates	NOT INDICATED	Not supplied	
	APEC	NOT INDICATED	Not supplied	
	Dichlorophenol	NOT INDICATED	Not supplied	Sanachem
	Dimethylphenol	NOT INDICATED	Not supplied	
	Pentachlorophenol	Almost insoluble in water	LD50 (oral, rat) = 27 mg/kg	Sanachem
	Alkyl phenols : pentaphenol to nonylphenol	Individual variation	Not supplied	
	1,1 - Dimethylethyl-4-methoxy-phenol (BHA)	Insoluble in water	Not supplied	
	Nonylphenol	Practical insoluble in water	LD50 (oral, rat) = 1300 mg/kg and 1620 mg/kg	Sasol
	o' Phenylphenol	Practically insoluble in water	Not supplied	Not indicated - Sasolburg
	Octylphenol	NOT INDICATED	LD50 (oral, rat) = 4 gm/kg	
Pharmaceutical products	o,p' DDD	Practically insoluble in water	LD50 (oral, rat) = 113 mg/kg	Polifin - Sasol
	Adiol	NOT INDICATED	Not supplied	
	Caffeine	Soluble in water	Not supplied	
	Cimetidine	Soluble in water	Not supplied	
	Clenbuterol	Very soluble in water	Not supplied	
	Cycloheximide	Slightly soluble in water	Not supplied	
	Ginseng	NOT INDICATED	Not supplied	
	Mifepristone (RU 486)	NOT INDICATED	Not supplied	
	p-Hydroxy benzoic acid	Slightly soluble in water	Not supplied	
	Synthetic estrogens e.g. Diethylstilboestrol	Insoluble in water	LD50 (oral, rat) = >3 gm/kg	
	Ethinylestradiol	Practically insoluble in water	LD50 (oral, rat) = >10gm/kg	
	Mestranol	Practically insoluble in water	Not supplied	
	Estradiol benzoate	Soluble in water	Not supplied	
	Estradiol valerate	NOT INDICATED	Not supplied	
	Estradiol phenylpropionate	NOT INDICATED	Not supplied	
	Conjugated estrogens	Water soluble	LD50 (intraperitoneal, rat) = 325 mg/kg	
	Prednisolone	Very slightly soluble in water	Not supplied	
	EPA (5,8,11,14,17 - Eicopentaenoic acid)	Is an oil - thus insoluble in water	Not supplied	
Endogenous estrogens	Enterodiol	NOT INDICATED	Not supplied	
	Estrone	Almost insoluble in water	Not supplied	
	Estradiol	Almost insoluble in water	LD50 (subcutaneous, rat) = >300mg/kg	
Plant substances containing estrogen-like compounds	Anthocyanidins	NOT INDICATED	Not supplied	
	Chalcones	NOT INDICATED	Not supplied	
	Coumestrol	Insoluble in water at acidic/neutral pH	Not supplied	
		Springly soluble in water at alkaline pH	Not supplied	
	Enterolactone	NOT INDICATED	Not supplied	
	Equol	NOT INDICATED	Not supplied	
	Flavonols	NOT INDICATED	Not supplied	
	Flavones	Insoluble in water	Not supplied	
	Flavonones	NOT INDICATED	Not supplied	
	Isoflavones	Individual variation	Not supplied	
	Phloretin	Insoluble in water	Not supplied	
	Phytoestrogens e.g. coumestrol	See coumestrol	Not supplied	
	genistein	Insoluble in water	Not supplied	
	saisolidine	NOT INDICATED	Not supplied	
	saisoline	Slightly soluble in water	Not supplied	
	saisolinol	NOT INDICATED	Not supplied	
	Ricinus communis var. minor (seed)	NOT INDICATED	Not supplied	
	Soybeans	NOT INDICATED	Not supplied	
	Soy	See Soybeans	Not supplied	
	Zearalenone	Insoluble in water	Not supplied	
	Aliquots of the extracts tested for total estrogen content of the Medicago Sativa plant (alfalfa plant)	NOT INDICATED	Not supplied	

3 Martindale (1993: 1166-1170, 1183-1184, 1187-1188, 1192-1193, 1195-1196).

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* Compiled from Budavari, S. et al., (1989:246-387), Weast et al. (1982:A1-1-27)

Classification	Estrogen / estrogen mimic	Water solubility *	Toxicity (LD50 and LC50 values)	Local Man
Naturally occurring substances that contain estrogen-like substances	Boron	NOT INDICATED	Not supplied	
	Broccoli	Not applicable	Not supplied	
	Cholesterol	Insoluble in water	Not supplied	
	Immunoglobulin G (IgG)	NOT INDICATED	Not supplied	
	Milk from pregnant cows	Soluble in water	Not supplied	
	Mycotoxins	NOT INDICATED	Not supplied	
	Phytohormones	Individual variation	Not supplied	
	Resorcylic acid lactones	Soluble in hot water	Not supplied	
	Xenobiotics	NOT INDICATED	Not supplied	
	Xenoestrogens (foreign estrogens)	Individual variation	Not supplied	
Miscellaneous	1-Hydroxychloridene	NOT INDICATED	LD50 (oral, rat) = 2400 mg/kg	
	Benzo(a)pyrene	Practically insoluble in water	Not supplied	
	DDT	See DDT	Not supplied	
	Dicofol	Insoluble in water	LD50 (oral, rat) = 1660 mg/kg	Sanachem - Durban
	Poly aromatic hydrocarbons (PAH's)	Individual variation	Not supplied	
	Tetrahydro-isoquinolines (TIQ)	Individual variation	Not supplied	
	PDDF's	Individual variation	Not supplied	
	Estrogens found in sewage water	Individual variation	Not supplied	
	Endrin	NOT INDICATED	LD50 (oral, rat) = 3 mg/kg	
	3 Adihydroxybenz(a)anthracene	NOT INDICATED	Not supplied	
Miscellaneous (continue)	Aromatic hydrocarbons	Individual variation	Not supplied	Not indicated - Sasolburg
	Carboxylic acid	Not specified	Not supplied	
	Chicken manure	Practically insoluble in water	Not supplied	
	Chlorinated hydrocarbons	Individual variation	Not supplied	Not indicated - Sasolburg
	Chlorinated organic compounds e.g. atrazine	see atrazine	Not supplied	
	chloridene	see chloridene	Not supplied	
	DDT	see DDT	Not supplied	
	endosulfan	see endosulfan	Not supplied	
	kepone	see kepone	Not supplied	
	methoxychlor	see methoxychlor	Not supplied	
	some PCB's	See individual PCB's	Not supplied	
	Chlorine compounds that bleach paper	Individual variation	Not supplied	Sappi Fine Papers - Stangermill
	Clone ID5	Not indicated	Not supplied	
	Crops grown in animal pastures	Insoluble in water	Not supplied	
	Dibutyl benzene-1,2-dicarboxylate (DBP)	Soluble in ca 2500 parts of water	Not supplied	
	Bis (ethylnesyl)phthalate (DEHP)	NOT INDICATED	Not supplied	
	Dimethylbenz(a)anthracene (DMBA)	Insoluble in water	LD50 (oral, mouse) = 340 mg/kg	
	Epidermal growth factor (human)	NOT INDICATED	Not supplied	
	Manure of swine, goats and cows	Practically insoluble in water	Not supplied	
	Milk	Soluble in water	Not supplied	
	Musk ketone (scent)	NOT INDICATED	Not supplied	
	Musk xylene (scent)	NOT INDICATED	Not supplied	
	Organochlorines	Individual variation	Not supplied	
	Poly chlorinated dibenzo-p-dioxins (PCDD)	Individual variation	Not supplied	Sanachem - Durban
	Petroleum byproducts	Individual variation	Not supplied	
	Tetra chloro-dibenzo-dioxins (TCDD)	NOT INDICATED	LD50 (oral, rat) = 20 ug/kg	
	Tetrachlorodibenzo-p-dioxin equivalents (TCDD-EQ)	Individual variation	Not supplied	

to be tested for in the South African water environment

re	Eliminate	Reason for elimination	Sample site	Codes for sampling areas
	Yes	Not likely to be found in water		Boskop dam = BKD
	Yes	Not likely to be found in water		Hartebeespoort dam = HBPD
	Yes	Not likely to be found in water		Rooideplat dam = RPD
	Yes	Not likely to be found in water		Vaalhartsirrigation scheme = VHIS
	Yes	Not likely to be found in water		Vaalriver (Vereniging) = VR (VEREEN)
	Yes	Not likely to be found in water		Vaal triangle (Sasolburg area) = VT (SBA)
	Yes	Not likely to be found in water		Bloemspruit Works = BSW
	Yes	Not likely to be found in water		Memel sewage works = MSW
	Yes	Not likely to be found in water		Windhoek sewage works = WSW
	Yes	Not likely to be found in water		Bloemfontein = BLOEM
	Yes	Not likely to be found in water		Ourban = DBN
	Yes	Not likely to be found in water		Cape Town = CT
	Yes	Not able to assay		Johannesburg = JHB
	Yes	Sampling point unknown		Pretoria (Northern & Southern areas) = Pta (N&S)
	No		Sanachem (Dbn)	Pietersburg sewage works = PSW
	No		Sanachem (Dbn)	Sasol = SAS
	Yes	To broadly described		
	Yes	Not able to assay		
	Yes	Analysis to expensive for this study (includes dioxin)		
	No		BSW, MSW, WSW, BLOEM, VEREEN, DBN, POTCH, CT, JHB, PTA (N&S), PSW	
	Yes	LD50 value = large, sampling point unknown		
	Yes	Sampling point unknown		
	Yes	To broadly described		Boskop dam = BKD
	Yes	Not likely to be found in water		Hartebeespoort dam = HBPD
	Yes	Not likely to be found in water		Rooideplat dam = RPD
	Yes	To broadly described		Vaalhartsirrigation scheme = VHIS
	No			Vaalriver (Vereniging) = VR (VEREEN)
	Yes			Vaal triangle (Sasolburg area) = VT (SBA)
	No			Bloemspruit Works = BSW
	No			Memel sewage works = MSW
	No			Windhoek sewage works = WSW
	Yes			Bloemfontein = BLOEM
	No			Potchefstroom = POTCH
	Yes	To broadly described		Cape Town = CT
	Yes	To broadly described-includes dioxin		Johannesburg = JHB
	Yes	Not likely to be found in water		Pretoria (Northern & Southern areas) = Pta (N&S)
	Yes	Not likely to be found in water		Pietersburg sewage works = PSW
	Yes	Not able to assay		Sasol = SAS
	Yes	Not able to assay		
	Yes	insoluble in water, sampling point unknown		
	Yes	Not likely to be found in water		
	Yes	Not likely to be found in water		
	Yes	Not likely to be found in water		
	Yes	Not likely to be found in water		
	Yes	Not likely to be found in water		
	Yes	To broadly described		
	Yes	Analysis to expensive for this study		
	Yes	Unlikely to contaminate water		
	Yes	Analysis to expensive for this study		
	Yes	Analysis to expensive for this study		