THE FEASIBILITY OF A HEALTH RISK ASSESSMENT FRAMEWORK TO DERIVE GUIDELINES FOR OESTROGEN ACTIVITY IN TREATED DRINKING WATER

Report to the
Water Research Commission

by

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Executive Summary

Background

Globally, endocrine disrupting chemical (EDC) research has been ongoing for the last 20 years. Since 1998, the Water Research Commission (WRC) of South Africa has looked at endocrine disrupting compounds in water. They have participated with the Global Water Research Coalition (GWRC), amongst others, in pro-active research on their effects and implications.

Most of the research done to date has focused on the reproductive effects and endpoints of endocrine disrupting chemicals in the environment, with a few investigations on effects on the immune system and thyroid function. South African waters (both treated and raw waters) have recently been found to have oestrogenic activity present in varying concentrations. Internationally, countries are in agreement that precautionary action should be taken. At this stage we are not certain at which levels endocrine disruptors adversely affect health, although we do have an indication that adverse effects occur.

A previous WRC report (Genthe and Steyn, 2008) proposed a framework to deal with endocrine disrupting chemicals and protect human health until it is possible to derive more specific guidelines for drinking water in South Africa. The proposed South African Framework is consistent with the precautionary principle as well as the South African approach to derive risk based water quality guidelines. The proposed framework recommends a precautionary risk-based approach whereby it derived a trigger value for oestrogen activity. The framework also suggests a tiered approach to screening and testing of chemicals in the water environment rather than testing for specific target chemicals. It further recommends that any results of oestrogen activity exceeding the recommended trigger value is a cause for further investigation and testing of the water is then necessitated.

A need to determine the feasibility of such a framework to derive guidelines for treated drinking water was therefore identified. It became necessary to test whether the tools and necessary organisational structures were available if testing revealed results exceeding the trigger value which implies further tiered testing of water samples.

Aims and objectives

- To determine whether the proposed framework (Genthe and Steyn, 2008) for endocrine disruptor chemicals is feasible for South African drinking water;
- To determine criteria to use for test inclusion in the proposed framework for endocrine disruptors in water;

- To evaluate the selected battery of tests and evaluate the applicability and feasibility of these tests for determining the endocrine disrupting capabilities with regards to reproductive impacts; and
- To investigate the international status quo with regards to tools for assessing endocrine disrupting techniques other than reproductive effects, i.e. immunological, neurological, cognitive and metabolic effects.

Results and Discussion

- **AIM 1: To determine whether the proposed framework for endocrine disruptor chemicals is feasible for South African drinking water**

The primary aim of this study was to assess the feasibility of the suggested framework for guidelines for endocrine disruptors in drinking water. The suggested approach involved the use of a trigger value of oestrogenic activity using bio-assays. The trigger value itself and its practicability was investigated to test its feasibility and to identify any potential problems. Workshops were held to share current knowledge and ideas regarding endocrine disrupting chemicals and how to deal with them in terms of drinking water quality management and protection of human health. The ultimate aim was to discuss practices and methods on how to deal with these chemicals in terms of early warning systems, how our knowledge can assist in the interim using a trigger value as a water quality guideline. Attendees were chosen to represent both government and researchers involved in endocrine water research. Researchers and scientists shared information regarding the limitations and difficulties in detecting EDCs, with the government representatives presenting the issues and difficulties involved in developing water quality guidelines.

The trigger value was based on a WHO (World Health Organisation) value of oestrogenic equivalency factor or quotients (EEQ). The standard for measuring oestrogenic activity is 17 β-oestradiol and the activity of all other compounds are measured against this.

A variety of environmental and drinking waters were tested for the EEQ ng/ℓ to assess the achievability and practicality of the proposed trigger value. These waters included river water sampled from and stored in plastic containers, household taps and springs, as well as tap water from conventionally treated drinking water. The trigger value of 0.7 ng EEQ/ℓ was exceeded in many cases where plastic drums were sampled. This is not surprising as the bio-assays are highly sensitive and the sampling procedure specifies all prevention of contact with plastic[1]. River waters

[1] Not all people in South Africa have access to treated tap water in their homes and therefore store water in plastic containers. The researchers investigated whether storage in plastic containers would increase the oestrogen activity of the water.

generally contained oestrogenic activity above the trigger value of 0.7 ng EEQ/ℓ. Most drinking waters from taps or straight from the distribution system contain below the 0.7 ng EEQ/ℓ.

Data from the literature was also investigated to examine the feasibility of the trigger value for drinking waters. For instance, Slabbert et al. (2008) looked at oestrogenic activity in South African source waters and their corresponding conventionally treated drinking waters. Final drinking waters ranged between less than the detection limit to a maximum of 0.18 ng EEQ/ℓ. These are well below the trigger value with all its safety factors and would not require additional investigations based on reproductive endocrine effects.

The results were within the range anticipated and it indicated that the South African framework making use of the trigger value is feasible. River waters generally contained above trigger value levels of oestrogenic activity and conventionally treated drinking waters were found to be below the trigger value. On those occasions where the trigger value was exceeded, additional situational analysis might explain the possible source of the oestrogenic activity and if this is not possible, then additional targeted chemical analyses should be conducted to determine the source.

The trigger value approach is considered feasible with the present limitations of testing for specific and individual chemicals. The available bio-assays are sensitive enough and are able to detect levels of EEQs many orders of magnitude below the recommended trigger value. Distinctions were observed in EEQs between different water types, with treated tap water having low EEQs compared to some river waters and tap waters stored in plastic containers.

- **AIM 2: To determine criteria to use for test inclusion in the proposed framework for endocrine disruptors in water**

Biological methods, also known as bio-assays, are becoming increasingly popular as screening tools because the specific chemical nature of an environmental sample is not always known. Bio-assays measure total oestrogenic and androgenic activity resulting from all the endocrine disrupting chemicals present in a water body, including unknowns.

No single assay can accurately predict the total oestrogenic activity of complex samples to all organisms. Both biological (*in vivo* and *in vitro*) and biochemical (*in vitro*) methods are used to determine endocrine disrupting chemicals' activity and effects.

The selection of the appropriate and relevant method is of crucial importance when conducting research on endocrine disrupting chemicals (American Waterworks Association Research Fund (AWWARF) / Global Water Research Coalition (GWRC), 2005). Therefore, the need exists to develop a recommendation for a suite of suitable and reliable methods available for conducting this analysis.

Additional projects, such as the GWRC toolbox project "In vitro bio-assays to detect oestrogenic activity in environmental waters" (2006) and the WRC's project currently being addressed "The compilation of a toolbox of bio-assays for detection of oestrogenic activity in water" (WRC 1816) are projects that look at the various options available to decide which test methods should be recommended. These research projects have investigated the most appropriate techniques for assessing endocrine disruption in water and include both *in vitro* and *in vivo* bio assays. This toolbox not only describes the assays available to date, but also summarises the advantages and disadvantages of the different methods.

- **AIM 3: To evaluate the selected battery of tests and evaluate the applicability and feasibility of these tests for determining the endocrine disrupting capabilities with regards to reproductive impacts**

Studies showed that some of these techniques are sufficiently advanced that they can be used as a cost-effective first-pass detection system, as well as together with other standard analytical methods, to measure oestrogenic pollutants in environmental waters. A major shortcoming is the lack of standardization for data analysis or interpretation. This was identified as a crucial step forward towards accurate bio-assay-derived oestrogenicity measurements.

The following shortcomings have been identified for the use of these bio-assays:

- **Cost**: Running costs were in the order of R2000 per sample at the time of writing in 2009.
- **Time** needed to complete a test. The assays are labour intensive. At present a laboratory with one technician is only able to process four samples per week. This severely limits recommending routine testing.
- **Capacity** is limited in the country. There are only a few experienced and trained individuals capable of carrying out these tests as well as few laboratories available with the necessary equipment to conduct the bio-assays. At the time of writing (2009), there were two active laboratories at universities set up to conduct these bio-assays on a routine basis. Other universities and water laboratories may be capable of carrying out the test with early warning to allow enough time to set-up and prepare for analysis.
- One of the problems in using *in vitro* assays to analyse environmental water samples is the presence of cytotoxic compounds.

Standard operating procedures (SOPs) would be required for sample collection, transport, and analysis of water for oestrogen activity. While some the SOPs have been developed, it has not been finalised. More importantly is the need for SOPs for interpreting the results. This has neither

been achieved internationally nor standardised yet[2].

EEQs are calculated in different ways depending on the laboratory doing the analyses. This is an issue that will need to be standardised, and has been recognised by the University of Pretoria and US EPA. One method of calculating EEQ measures the mean maximum response of the positive control which is then set as 100% and all other samples are expressed relative to this maximum response in the same assay. This method to calculate EEQs was preferred to other methods in which the relation between the EC50 of the standard substance and the EC50 of the sample are reported as EEQ.

- **AIM 4: To investigate the international status quo with regards to tools for assessing endocrine disrupting techniques other than reproductive effects, i.e. immunological, neurological, cognitive and metabolic effects.**

Advances in methodologies have now been made with regard to testing for thyroid activity (OECD, 2006). Tests are close to being standardised, similar to the process that oestrogenic activity bio-assays went through in the GWRC/WRC study (2008).

Conclusions

From the results it is evident that the proposed South African framework to assess endocrine disrupting activity in water and compare it with a trigger value is feasible. If the trigger value is exceeded, the possible cause or source would need to be identified. A multidisciplinary team would need to be assembled to look at the possible sources such as industry, agriculture, waste streams, etc. and follow-up samples would need to be taken to identify the specific chemicals responsible, before remedial action could be taken.

Although much progress has been made in terms of identifying bio-assays to assess the presence of oestrogen activity in our drinking waters, more research is needed to build the capacity within the country. Research should concentrate on decreasing the cost and timing of these assays while standard operating procedures should be developed for the current set of *in vitro* and *in vivo* assays. Bio-assay research should also focus on developing more *in vivo* methods to allow for

[2] A range of guideline documents are being compiled within the WRC project "The Manual of guidelines for projects on EDCs in water resources". This manual is divided into different volumes as follows:

Volume 1: Monitoring and assessment guide – Prof James Meyer .... continues

Volume 2: Sampling guide – Dr Ralph Heath

Volume 3: Toolkit / Methods – Prof Tiaan de Jager

Volume 4: Management Options – Dr Ralph Heath

transgenerational capabilities and whole organism metabolism. Additional research will need to examine effects on thyroid function, immunosuppression and effects on neurodevelopment.

Recommendations for Future Research

Most drinking water treatment facilities will not be in a position to test for oestrogenic or other endocrine activity due to either lack of capacity or for financial reasons. From the results, it is clear that of the waters tested, most treated drinking waters appear to contain less than the recommended trigger value for oestrogenic activity at the tap and would not require specific additional investigation. One can therefore expect that a large proportion, if not all of the endocrine disrupting chemicals, will be removed in a properly functioning drinking water treatment works. If this is not achieved, more advanced processes can be used to achieve greater removal efficiency.

- It is recommended that, as a way forward, information regarding the local conditions should be collected in terms of size, functioning, and type of treatment processes involved at each of these treatment facilities. This is comparable to a qualitative risk assessment on the potential human health risks from industry and agriculture where treatment facilities would not be able to effectively remove these substances from the water, potentially posing human health risks. This could support water safety plans, as recommended by WHO, that currently need to be implemented by each municipality.
- Local conditions should then be compared to the Snyder (2003) report to assess whether our treatment facilities are capable of removing endocrine disrupting activity from our waters. This might be more feasible approach than to have all treatment facilities undergo testing of their waters and it would also be more cost-effective.
- In addition to the above information, information regarding the different agricultural activities to qualitatively assess the potential for pesticide use and other endocrine disrupting chemicals as well as the different industries in South Africa should be listed and mapped.
- GIS interactive electronic maps of this information could then be designed to make the effort more user-friendly.
- As a first screen, a simple enzyme-linked immunosorbent screening assay (ELISA) for a standard hormone (oestrone) as described in Swart and Pool (2007) could be used to screen the water treatment works throughout the country for their efficiencies in the removal of hormones.
- Attention should be given to other endpoints of endocrine disrupting chemicals such as that of the thyroid, followed by activity related to immune system activity and neuro-behaviour.

Exceedence does not imply health risk. The trigger value does not imply a health risk if it is exceeded. It only implies that further investigation, testing and analyses should be initiated. The implication is that there are endocrine disrupting chemicals, more specifically oestrogen activity in the water and that this may have some health impacts, the exact effects thereof still unclear. This is the precautionary approach to not turn a blind eye but to rather implement a process to prevent health impacts in future.

Capacity Development

The original capacity development planned for the project could not take place as the student at the University of KwaZulu-Natal left the university towards the middle of the project due to health reasons. Capacity development took place with one female researcher, Maronel Steyn, being exposed to the process of guideline development within the risk assessment process used within the guideline development process and understanding the endocrine system.

Archiving of Data

No data with the exception of that given in the report was created in the execution of this project.

Conference Presentation and Proceedings

Genthe, B and Steyn, M. 2008. Potential health risks associated with using dam water for domestic purposes in an urbanised area. WISA, Sun City, 18-22 May 2008.

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1. Background: Endocrine Disruptors in Drinking Water

Globally, endocrine disrupting chemical (EDC) research has been ongoing for the last 20 years. Since 1998, the Water Research Commission of South Africa, in collaboration with the Global Water Research Coalition (GWRC), has been pro-active in undertaking research on endocrine disrupting compounds in water.

Chemicals considered to be endocrine disruptors have been found in South African waters and wastewaters in various studies (Aneck-Hahn et al., 2002, 2008, 2009; Burger et al., 2008; Dalvie et al., 2003; Bornman et al., 2005; Slabbert et al., 2007; Bornman et al., 2008; Slabbert et al., 2008). A priority list of suspected endocrine disrupting chemicals was compiled by the Department of Water Affairs and Forestry (DWAF). Of these, thirty-three (33) were listed as potential endocrine disrupting chemicals that are frequently used in South Africa and occur in different water bodies around the country (KV 143/05).

As part of the Endocrine Disrupting Compounds Research Programme (Burger et al., 2008), the WRC has funded numerous research projects dealing with endocrine disrupting chemicals in the environment. This included projects dealing with development and standardisation of testing methodologies capable to determine the presence of these chemicals in water. In addition, attention has also been given to address the potential human health risks associated with the presence of these chemicals in drinking water.

The list below is not exhaustive for endocrine disrupting research supported by the Water Research Commission, but provides an indication of related research:

- Burger AEC. 2005. WRC programme on endocrine disrupting compounds (EDCs): Volume 1. WRC report number KV 143/05.
- MS Bornman, HJ van Vuren, H Bouwman, C de Jager, B Genthe, EJ Barnhoorn. 2007. Endocrine disruptive activity and the potential health risk in the urban nature reserve (EDC). WRC report number 1505/1/07.
- Slabbert JL, Venter EA, Moletsane M, Van Wyk JH, Blaise C and Aneck-Hahn NH. 2008. An investigation of the oestrogenic activity in water from selected drinking water treatment processes. WRC report number 1532/1/08.
- Genthe B and Steyn M. 2008. Health Risk Assessment Protocol for Endocrine Disrupting Chemicals (EDC). WRC report number KV 206/08.
- Burger AEC. 2008. WRC Research programme on Endocrine Disrupting Compounds (EDCs): Volume 2. Implementation of a research programme for investigating EDCs in SA Water systems. WRC report number 1402/1/08.

- De Jager C, Aneck-Hahn NH, Barnhoorn IEJ, Bornman MS, Pieters R, Van Wyk JH, Van Zijl C. In Progress. The Compilation of a Toolbox of Bio-assays for Detection of Oestrogenic Activity in Water. WRC report number 1816 (unpublished).

The purpose of this study is to determine the feasibility of the proposed framework described in detail in WRC Report KV 206/08 (Genthe and Steyn, 2008). The aims of the project are based on the recommendations subsequent to the report and are as follows:

- To determine whether the proposed framework (KV 206/08) for endocrine disruptor chemicals is feasible for South African drinking water;
- To determine criteria to use for test inclusion in the proposed framework for endocrine disruptors in water;
- To evaluate the applicability and feasibility of the selected battery of tests for determining endocrine disrupting activity with regards to reproductive impacts; and
- To investigate the international status quo with regards to tools for assessing endocrine disrupting techniques other than reproductive effects, i.e. immunological, neurological, cognitive and metabolic effects.

For purposes of clarity, this report summarises the South African Framework proposed for endocrine disrupting activity in drinking water. It highlights the risk assessment approach as well as the derivation of a trigger value described in detail in the KV 206/08 report. Thereafter the report addresses each of the study aims.

The primary focus though is the testing of the recommended approach to handle or manage oestrogen activity in drinking water. In addition, a summary description is provided on the methodologies available for screening for oestrogen activity in drinking water and shortcomings of the current bio-assays are highlighted. In conclusion, the report provides further recommendations and describes the way forward for dealing with oestrogen activity in the South African environment.

2. Introduction

Internationally, most of the research done to date has focused on the reproductive effects and endpoints of endocrine disrupting chemicals in the environment. This is also the case in South Africa. South African waters have recently been found to have oestrogenic activity present in varying concentrations (Bornman, 2007; Burger et al., 2008; Slabbert et al., 2008; Aneck-Hahn et al., 2009). Current international research focus on EDCs and human health has seen significant growth in non-genomic modes of action, and an increase in thyroid, immunological, neurological, and carcinogenic effects. This report focuses on aspects relevant to the reproductive system only.

One of the main questions currently facing scientists is: Do the levels of oestrogenic activity found in our drinking waters have the potential to cause harmful effects in humans?

Policy-makers on the other hand have to consider: When is there enough scientific understanding to proceed with actions? Scientists are often very conservative in declaring their findings with certainty. Raffensperger and deFur (1999) pertinently comment: "Scientists would rather make the mistake of not finding something that really exists". They continue sarcastically to say "Pesticides may damage reptiles, but it is helpful to have a lot of dead bodies before publishing".

3. Time for Precautionary Action

Internationally, countries are in agreement that precautionary action should be taken. The precautionary principle is sometimes referred to as "prudent avoidance"#[3] (Raffensperger and deFur, 1999). In terms of endocrine disrupting chemicals this approach means that if the negative impacts of these chemicals used in the environment are not yet known or cannot be proved yet, that the use of these chemicals are prohibited, until proven otherwise (Diamanti-Kandarakis *et al.,* 2009).

As part of the precautionary action in the EU, a group has been set up for the Regulation, Evaluation, Authorisation and Restriction of Chemicals (REACH) (http://ec.europa.eu/enterprise/sectors/chemicals/reach/index_en.htm) when chemicals are produced in excess of 1 tonne per annum. The US EPA established an Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) (http://www.epa.gov/endo/pubs/edspoverview/edstac.htm) and recommends a tiered approach to screening of target chemicals for endocrine disrupting properties. US Congress amended the Safe Drinking Water Act in 1996 and requires screening of drinking water sources for endocrine disruptors (http://www.epa.gov/endo/pubs/edspoverview/primer.htm).

Similarly, Environment Canada in 2003 (Hull and Swanson, 2006) adopted the weight-of evidence approach which relies on the most reliable information and recommends a tiered-testing approach. Australia proposed the use of the precautionary principle based on a no-observed-effect-level or NOEL (Stauber, 2002).

The proposed South African Framework (Genthe and Steyn, 2008) is consistent with the precautionary principle. The proposed framework recommends a precautionary risk-based approach whereby it derived a trigger value for oestrogenic activity. The framework also suggests a tiered approach to screening and testing of chemicals in the water environment rather than testing for specific target chemicals.

#[3] Prudence has the connotation of "good sense"

4. Summary of Proposed framework for guidelines for endocrine disruptors in drinking water

Endocrine disrupting chemicals occur in South African waters and a process is needed to respond without waiting for perfect data. At this stage we are not certain at which levels endocrine disruptors adversely affect health, although we do have an indication that adverse effects occur (Aneck-Hahn et al., 2002, 2008, 2009; Dalvie et al., 2003; Bornman et al., 2007; Slabbert et al., 2007; Bornman et al., 2008; Slabbert et al., 2008). The WHO has defined a "no observed adverse effect level", or NOAEL based on a value where no physiological effects were observed.

The WRC report KV 206/08 (Genthe and Steyn, 2008) describes the background literature to endocrine disrupting chemicals in water and the potential for human health effects. It addressed the development of guidelines for water as well as the uncertainty involved in applying the risk assessment methodology for endocrine disrupting chemicals since the data is not available to deal with all relevant endpoints of EDCs in water yet. The report proposes a framework to deal with oestrogen activity and protect human health until it is possible to derive more specific guidelines for drinking water[#4] in South Africa.

It suggested water samples be tested for endocrine disrupting activity rather than testing the water for individual targeted chemicals. One of the benefits of this is that people are seldom if ever exposed to only singular chemicals. We are mostly exposed to a mixture of chemicals, each eliciting different activity capabilities and affecting different endpoints within the human body. The report also proposed that the endocrine disrupting activity, more specifically the oestrogenic activity of a water sample be tested and the concentration compared to a trigger value. The report described the derivation of a trigger value for drinking water in detail. It further recommends that results of oestrogen activity exceeding the trigger value is a cause for further investigation and testing of the water is then necessitated.

It is acknowledged that the proposed trigger value approach described in KV 206/08 is not the ideal solution and has shortcomings. This was the first attempt to develop a framework to deal with these chemicals in drinking water in South Africa in order to protect against human health effects in the long term.

#[4] For purposes of this document, drinking water refers to treated municipal supply from taps. The report however also includes results of waters from rivers / streams as well as boreholes and water stored in containers prior to drinking, making provision for other sources of water that people drink.

5. Guideline Development for Endocrine Disrupting Chemicals

The Department of Water Affairs and Forestry is looking at refining, aligning, and expanding the current South African National Water Quality Guidelines of 1996. It is expected to be a multi-tiered assessment system based on a risk approach.

Guidelines are health-based targets normally set at levels safe for human consumption for continuous use. These targets should be reasonable in terms of acceptable risk, costs, country situation, and achievable by treatment methodology available in the country.

According to the World Health Organisation (WHO), the health risk assessment approach is the recommended process used to derive guideline values for substances in water (WHO, 2004). However, this process has been developed based on toxicity or carcinogenicity of chemicals and does not fit endocrine disrupting chemicals adequately, with further research needed.

An approach for managing endocrine disruptors in water is needed. A framework for guideline development for endocrine disrupting chemicals making use of a preventative approach was developed for South Africa based on the WHO risk assessment approach. The human health risk assessment process is described along with the uncertainties surrounding the current methodology. In addition, substitute approaches and methods are also discussed.

6. Healsath Risk Assessment Approach for Guideline Development

Health risk assessment is the process or method of determining if an activity (man-made or natural) will negatively impact humans. Risk assessment can therefore be used as a decision making tool, to support decisions that protect public health and the environment, such as guideline development.

Human health risk assessment involves a quantitative and/or qualitative process to characterise the nature and magnitude of the risks to public health from exposure to hazardous substances (Schwab and Genthe, 1998) and involves four distinct, but interacting phases (NRC/NAP, 1983), namely:

- Hazard Identification
- Dose-Response Assessment
- Exposure Assessment
- Risk Characterisation

In order to develop policy and legislation to protect humans and the environment from endocrine disruptors, it is first necessary to determine the risk to human health and the environment.

Although endocrine disrupting compounds cause serious concerns, standardised methodology on how to apply the current risk assessment methodology to assess the potential risk of developing endocrine disrupting effects is unavailable at this stage. The sections that follow explain the uncertainties involved in applying the current methodology to assess the health risks from exposure to endocrine disrupting substances.

There is no standardised method or guideline to assess human health risks associated with endocrine disrupting chemicals (WHO, 2004). Current human health risk assessments differentiate between risks from chemical substances that cause carcinogenic (causing cancer) or toxic (non-carcinogenic) effects (Zala and Penn, 2004).

It is general practice in health risk assessments to assume that toxic substances have some safe level (non-zero threshold) at which no adverse health effects will occur over a lifetime of exposure to the substance (US EPA, 2002; WHO, 2004). This safe threshold is also referred to as the reference dose which is derived from an acceptable daily intake (ADI).

Carcinogenic (cancer-causing) substances, on the other hand, are assumed to have no safe level of exposure (US EPA, 2005; WHO, 2004). This means that it is assumed that an exposure to even a very small amount of carcinogen will result in a potential risk and slope factors are therefore used as opposed to reference values.

Instead of using a linear dose-response curve and extrapolating effects at low doses, endocrine disrupting chemicals generally follow either a U-shaped or inverted U-shaped dose-response curve, also known as a non-monotonic dose-response relationship. Welshons et al (2003) found that endocrine disrupting chemicals are biologically active at low environmentally relevant doses. This implies that chemicals considered safe at medium doses, could have adverse effects at lower doses (Lyons, 2003). When following a U-shaped response curve, the strongest responses are found at low and high concentrations (Zala and Penn, 2004). For the inverted U-shape, responses disappear at high exposures.

Based on various research studies completed internationally, it is believed that for some endocrine disrupting chemicals there are no thresholds. Even at extremely low doses, endocrine disrupting chemicals have been found to cause behavioural changes or other damaging effects. These low dose findings have led to a paradigm shift in the way toxicology studies are carried out (Sheehan, 2000; Welshons et al., 2003; Zala and Penn, 2004).

7. Effectiveness of Current Risk Assessment Methodology for Endocrine Disrupting Chemical Guideline Development

The following properties of endocrine disrupting chemicals influence the ability of the current methodology to assess the human health risks associated with these substances and have been discussed in the previous report (KV 206/8) in more detail, but are summarised here for the sake of clarity:

Epidemiological evidence – Population based epidemiological studies relevant to endocrine disruption are few and often limited by factors such as the time lag between exposure and clinical disease (Solomon and Schettler, 2000). It is difficult to establish dose-response relationships for human exposure of endocrine disruptors and incident disease, since everyone has been exposed to endocrine disrupting chemicals at sometime or the other and thus no control reference exists (Myers et al., 2003).

Threshold and linear model assumptions – As mentioned above, it has been found that endocrine disrupting chemicals follow either a U-shaped or inverted U-shaped dose response curve, which implies that even extremely low doses cause changes or have damaging effects.

Transgenerational properties – Endocrine disrupting chemicals can be transferred across the placenta and into maternal milk, thereby affecting the offspring (Zala and Penn, 2004). Developmental toxicity can result from exposure of either parent prior to conception from exposure of the embryo *in utero* or from exposure of the progeny after birth. *In vivo* studies on pregnant animals and their progeny have been widely used in developmental toxicity assessment (WHO, 2003). Developmental effects of endocrine disruptors tend to be latent, where traditional endpoints of toxicity may not be detectable until sexual maturity (Daston et al., 2003).

Timing of exposure or delayed effects – Endocrine disrupting chemical tests are often only done on adult animals, yet sometimes endocrine disrupting chemicals only affect early stages of development. In addition, the effects of exposure during early life stages may not manifest until adulthood. The pharmacokinetics of chemicals is markedly different during foetal and early postnatal life relative to adulthood (Den Voogt and Van Hattum, 2003). Similarly, pregnant and non-pregnant women also differ in this regard (Welshon et al., 2003).

Synergistic and additive effects between chemicals – Most toxicological studies examine the effects of only a single chemical at a time. Endocrine disrupting chemicals can act additively and even synergistically (Soto et al., 1994 cited in Kristensen et al., 1995; Carpenter et al., 1998; Vonier et al., 1996). There is already evidence that suggest that the exposure to several endocrine active substances may result in a combined response more than the threshold for effects, even

though individually each chemical is below its effect level (Silva et al., 2002 cited in MRC/IEH, 2004). Particularly worrying regarding environmental exposure was evidence suggesting that mixtures of weakly active endocrine disrupters could affect the activity of strong (endogenous) hormones and that some chemicals, not themselves hormonally active) may promote the oestrogenic activity (MRC/IEH, 2004). Thus, chemicals that have been declared safe could still be harmful because individuals are often exposed simultaneously to many different pollutants (WHO, 2003; Zela and Penn, 1998).

Evidence of effects and end-points – It is essential that the correct end-point be examined when conducting the dose-response assessment of a chemical thought to be an endocrine disruptor (Mantovani et al., 1999; Mantovani, 2002). Molecular mechanisms of endocrine disrupters could help to classify which compounds need to be removed from the environment. Histopathological data is an important tool to assess the toxic affects on for example male reproductive organs, since chemicals with oestrogenic or anti-androgenic activity may have reproductive effects in males (Royal Society, UK, 2000).

8. The Trigger value approach

The previous report (KV 206/08 "Health risk assessment protocol for endocrine disrupting chemicals") suggested a framework for endocrine disrupting chemical guidelines for drinking water for South Africa based on a tiered approach. First level screening tests for reproductive endocrine disrupting capability was recommended rather than testing specific or individual chemical concentrations. Screening using a battery of *in vitro* and *in vivo* tests quantitatively expressing the results of oestrogen activity of a water sample containing a mixture of chemicals in terms of their relative potency is recommended. An approach similar to toxic equivalency factors can be used for hormones and their activity in water and can be expressed in terms of equivalency factors. A value above which a more detailed assessment is recommended is the "trigger value".

If endocrine disruption is detected at concentrations higher than a specified "trigger value", then a more detailed assessment was recommended to identify the chemicals responsible.
The primary aim of this study was to assess the feasibility of the suggested framework for guidelines for endocrine disruptors in drinking water.

The suggested approach involved the use of a trigger value of oestrogenic activity using bio-assays. The trigger value itself and its practicability was looked at to test its feasibility and to identify any potential problems.
The trigger value was based on a WHO (World Health Organisation) value of oestrogenic equivalency factor or quotients (EEQ). The most potent form of oestrogenic activity is 17 β-oestradiol and all other compounds with activity are measured against this. See table 1 below.

Table 1: Relative potency of some oestrogenic chemicals (Legler et al., 1999; Ghijsen and Hoogenboezem, 2000)

Substance	Relative potency to 17β-oestradiol
17β-oestradiol	**1**
Oestrone	$5.8X10^{-2}$
17 α-oestradiol	$1.6X10^{-2}$
Bisphenol A	$7.8X10^{-6}$
di-n-butylphthalate	$1.8\ X10^{-8}$
Dimethyl phthalate	$1.1X10^{-5}$
n-nonyl phenol	$3.8X10^{-5}$
n-octyl phenol	$1.4X10^{-6}$
o,p-DDT	$9.1X10^{-6}$
Dieldrin	$2.4X10^{-7}$

An acceptable intake was calculated based on known induction of hormone changes and equated to intake rates. The value was converted to exposure of oestrogenic activity via water intake with a number of safety or modifying factors included and is described in more detail in the following section.

8.1 Calculation of equivalent trigger value

The equation to calculate the trigger value is as follows:

$$GuidelineValue = \frac{(ADI * bw * P)}{IR}$$ (WHO, 2004)

Where:

ADI = acceptable daily intake;

bw = body weight;

P = portion of exposure allocated to water;

IR = intake rate of water

The acceptable daily intake or ADI has been calculated by the WHO as 50 ng/kg body weight per day. Based on the proposed risk assessment framework to derive guideline values, the target value, or in this case, the trigger value is based on the acceptable daily intake. This value is derived by applying a number of uncertainty factors to the toxicity data to take into account 1) differences in sensitivity to toxic effects within and between species, and 2) differences in toxic

effects between chronic and sub-chronic exposure. This may be in the order of 1 up to 10 000 (WHO, 2004; IPCS, 1994). In the case of 17β-oestradiol the uncertainty factor used was 1000 (RIVM, 2004). The WHO derived an "acceptable daily intake" (ADI) of 50 ng/kg/d for 17 β-oestradiol. This ADI was based on induction of hormone changes in post-menopausal women who have low endogenous oestradiol production. These changes were induced at dose levels >5ug/kg body weight per day. To compensate for sensitive subpopulations, individual variation and % availability to induce response, a safety factor of 1000 was used. In addition, only a certain percentage will be available and another percentage will be unbound. This led to the suggested ADI of 200 pg/kg/d.

For water quality guidelines the WHO (2004) allocates a 10% exposure to water consumption where: the environmental concentrations of these chemicals in air, food, soil and water are not available intakes are estimated based on consideration of chemical and physical properties.

Most water quality guidelines use 10% as an allocation to account for additional exposure through other routes (e.g. inhalation and dermal absorption) (IPCS, 1994). This low proportion allocated to water as a source of pollution adds extra safety to the guideline (WHO, 2004) and now makes the safety factor 10 000.

The calculated trigger value is therefore as follows

$$TriggerValue = \frac{(200\,pgperkg * 65kg * 0.1)}{2\ell}$$

$$\cong \ 0.7\ ng/\ell$$

Although a trigger value was calculated, variations and uncertainty in the trigger value calculation were investigated assuming a range in ingestion volumes and body weight, with results shown below (Figures 1, 2 & 3 and Table 2). Monte Carlo analysis was performed assuming a normal distribution. The trigger value is expected to vary, with a 90% certainty, between 0.14 ng/ℓ and 3.44 ng/ℓ. The uncertainty analysis also showed that the volume consumed could range between 0.3 litres up to 7 litres for a 65kg adult, based on the assumption of a normal distribution.

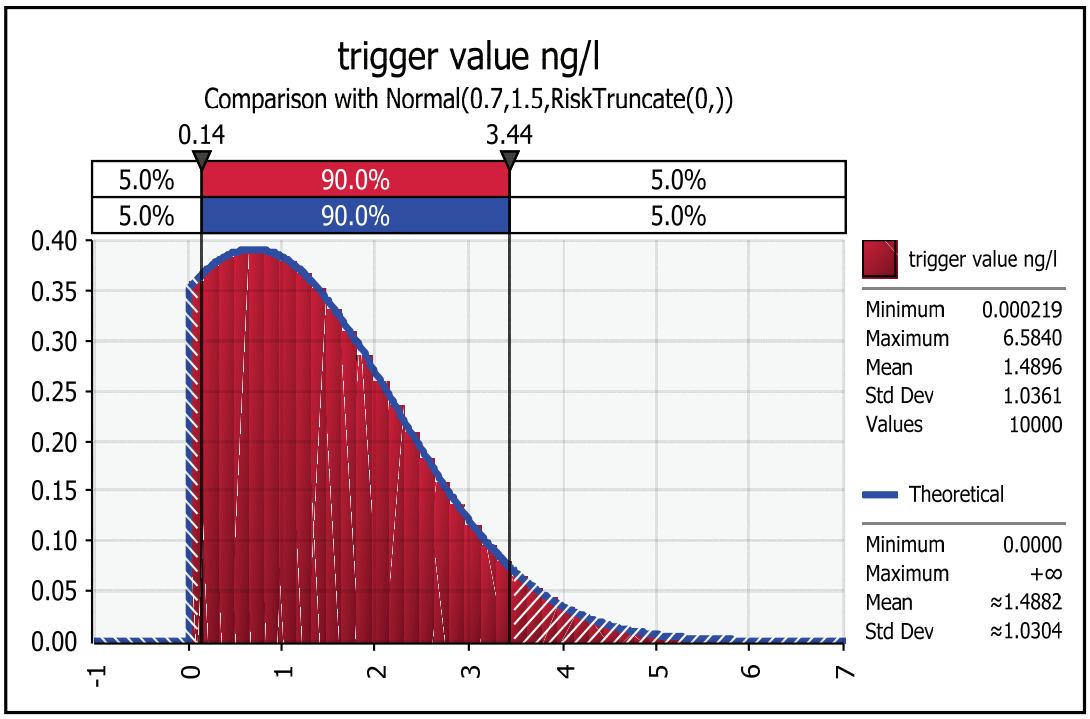

Figure 1: Uncertainty analysis for trigger value calculation (Monte-Carlo analysis)

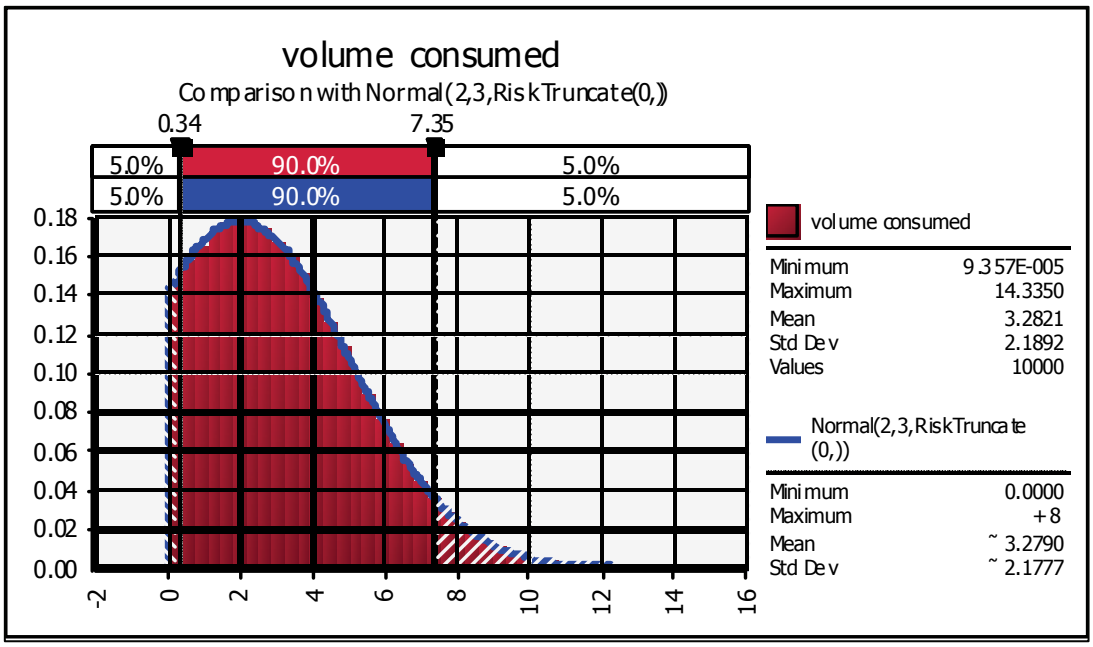

Figure 2: Uncertainty Analysis for volume consumed (Monte-Carlo analysis)

Table 2 shows the effect of a combination of assumed body weight and drinking water intake rate scenarios on the trigger value. It was considered to be unrealistic to have a 15kg child consuming more than 5 or 6 litres and was therefore not calculated. The 0.7 ng/ℓ EEQ is used as the average trigger value.

One must keep in mind that this trigger value is 1/10 000th of the no-observed adverse effect level (NOAEL) or no physiological response in the human body.

Table 2: Effect of body weight and intake rate on the trigger value

	Trigger value ng/ ℓ EEQ				
Volume ingested Body weight in kg	0.5 ℓ	1 ℓ	2 ℓ	5 ℓ	6 ℓ
80 kg		1.6	0.8	0.3	0.3
65 kg		1.3	0.7	0.3	0.22
15 kg		0.3	0.2	Not realistic	Not realistic
Infant-5 kg	0.2				

The effect of body weight and intake rates does not have a large effect as the recommended trigger values range from the lowest 0.2 ng/ℓ to 1.6 ng/ℓ EEQ.

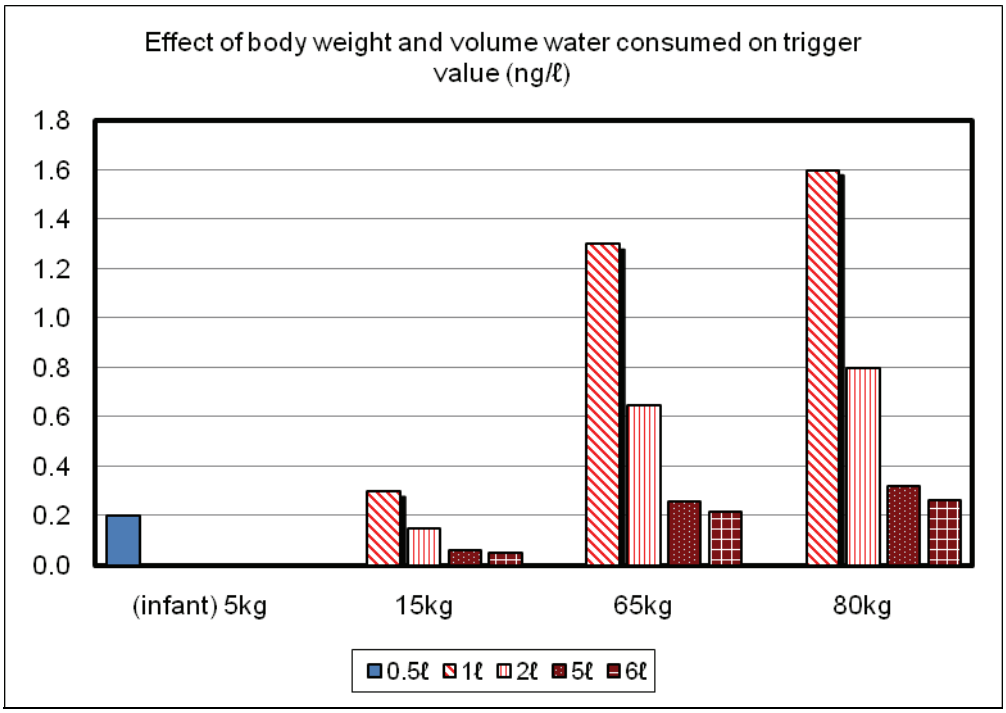

Figure 3: Effect of variations in body weight and volume water ingested on the calculated trigger value

8.2 Assessing the Trigger Value Approach

Workshops were held to share current knowledge and ideas regarding endocrine disrupting chemicals and how to deal with them in terms of drinking water quality management and protection of human health. The ultimate aim was to discuss practices and methods on how to deal with these chemicals in terms of early warning systems, how our knowledge can assist in the interim using a trigger value as a water quality guideline. Attendees were chosen to

represent both government, and researchers involved in endocrine water research. Researchers and scientists shared information regarding the limitations and difficulties in detecting EDCs, with the government representatives presenting the issues and difficulties involved in developing water quality guidelines.

8.3 EEQs for selected environmental and drinking water samples

A variety of environmental and drinking waters were tested for the EEQ ng/ℓ to assess the feasibility and practicality of the proposed trigger value (Table 3). These waters included river water sampled from and stored in plastic containers, household taps and springs, as well as tap water from conventionally treated drinking water. Environmental waters (although they are not considered to be drinking waters) were included in this assessment of feasibility to indicate whether the trigger value is exceeded and if so by what factor.

Table 3: Oestrogenic activity of a variety of environmental and drinking water samples expressed as oestradiol equivalents (EEQ) (ng/ℓ).

Description of water sample	No.	EEQ (ng/ℓ)	Fraction of trigger value 0.7 ng/ℓ or
Tap water – conventional treatment (A)	1	Below detection limit	-
Tap water – conventional treatment (B)	2	Below detection limit	-
Tap water – conventional treatment (C)	3	0.3	0.3/0.7 = 0.45
Mine water from tap	4	0.68	0.971
Borehole stored in plastic drum	5	1.82	2.60
Borehole water (private borehole)	6	1.30	1.86
Spring (and runoff from rain water)	7	2.29	3.27
Tank (large plastic tank)	8	Below detection limit	-
Borehole (privately owned borehole)	9	2.15	3.07
Spring (A)	10	2.48	3.54
Spring (B)	11	0.63	0.900
Borehole? Plastic drum	12	1.90	2.71
Communal tap (water pump)	13	0.83	1.19
Spring (C)	14	Below detection limit	-
River water	15	1.10	1.57
River water	16	10	14.3
River water	17	13	18.6
River water	18	10	14.3
River water near light industry	19	0.00095	0.00135
River water	20	0.00082	0.00117
River water	21	5.80	8.29
River water near light industry	22	0.013	0.0183
River water	23	2.4	3.43
River water close to wastewater effluent	24	0.024	0.0339

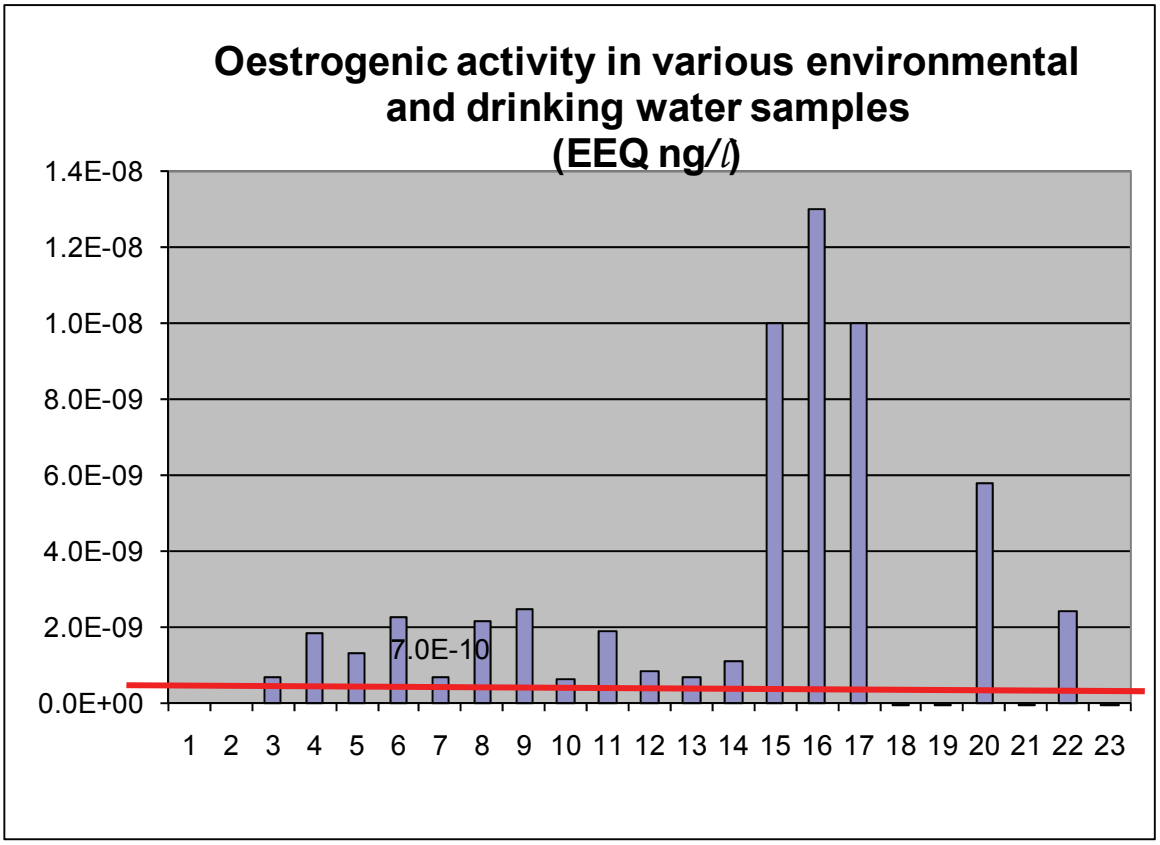

Figure 4: Oestrogenic activity in various environmental and drinking water samples in ng EEQ / ℓ

The trigger value of 0.7 ng EEQ/ℓ was exceeded in many cases where plastic drums were sampled (Figure 4). This is not surprising as the bio-assays are highly sensitive and the sampling procedure specifies prevention of contact with plastic. River waters generally contained oestrogenic activity above the trigger value of 0.7 ng EEQ/ℓ. Even though the trigger value was exceeded, this does not indicated that endocrine disrupting effects will occur as the trigger value is very conservative and precautionary being 1/10000th of the NOAEL with no physiological effects anticipated. The trigger value is meant to be used as a first screen early warning of potential oestrogen effects.

It was found that wastewater treatment works were not very successful in removing oestrogenic activity. In South Africa source waters were found to contain less than the detection limit to a maximum of ~0.8 ng EEQ/ℓ (Slabbert et al., 2008), which is close to the trigger value. The most recent study on wastewater treatment effluents found 11 ng EEQ/ℓ in the effluent (Bicchib et al., 2009). According to Kinnberg (2003) reviewing the international literature, wastewater treatment effluents were typically below 25 ng EEQ/ℓ with untreated surface waters generally in the order of 1-15 ng EEQ/ℓ. This illustrates that wastewater treatment effluents would require additional

investigation according to the recommended trigger value approach, but that drinking waters were generally within the recommended range.

Most drinking waters originating from conventional treatment systems or from taps contained below the 0.7 ng EEQ/ℓ. Data from previous studies was also investigated to examine how many of the water samples contained more or less than the recommended trigger value for drinking waters. For instance, Slabbert et al., (2008) looked at oestrogenic activity in South African source waters and their corresponding conventionally treated drinking waters. Final drinking waters ranged between less than the detection limit to a maximum of 0.18 ng EEQ/ℓ. These are well below the trigger value with all its safety factors and would not require additional investigations based on reproductive endocrine effects.

This also indicates the success of the recommendation of the trigger value. The results were within the range anticipated. River waters generally contained above trigger value levels of oestrogenic activity and conventionally treated drinking waters were found to be below the trigger value.

On those occasions where the trigger value was exceeded, additional situational analysis might explain the possible source of the oestrogenic activity and if that were not possible, then additional targeted chemical analyses should be conducted to determine the source.

As with all recommendations for managing uncertainties, there are a number of shortcomings in this recommended trigger value, and it must be remembered that the value is based on uncertainty factors taking into account variability between and within species.

9. Bio-assays for endocrine disruption activity in water

Bio-assays generally are significantly more sensitive than chemical methods. In addition, they provide a combination of potency and dose and, more importantly, they need no prior knowledge of the specific chemical nature of a sample. One of the major problems in controlling endocrine disruption in water is the number of possible chemical contaminants responsible as well as the complexity of the tests.

In order to assess whether endocrine disruptors are present in water one can do one of two things: one either carries out individual tests for each of the chemicals thought to have endocrine disruption capabilities as well as the potential to occur in a particular area under investigation, or test the water sample for endocrine disrupting activity using one or more of the available bio-assays.

The first option is not practical, as the general population is thought to be exposed to hundreds of endocrine disruptors. The latter option becomes more of a feasible option where one obtains

biological measures of exposure or biomarkers. This option is also in line with the DEEEP or "Direct Estimation of Ecological Effects Potential" approach which assesses the ecological hazard of complex effluents on freshwater systems (2003) followed by the National Toxicity Monitoring Programme that was initiated by DWAF.

Biological methods also known as bio-assays are becoming increasingly popular as screening tools because the specific chemical nature of an environmental sample is not always known. Bio-assays measure total oestrogenic and androgenic activity resulting from all the endocrine disrupting chemicals present in a water body, including unknowns. As the effects of chemical mixtures cannot always be elucidated from their concentrations, bio-assays are an important component of examining the presence of, and integrating the effects of complex mixtures of endocrine disrupting chemicals. No single assay can accurately predict the total oestrogenic activity of complex samples to all organisms. Both biological (*in vivo* and *in vitro*) and biochemical (*in vitro*) methods are used to determine endocrine disrupting chemicals activity and effects.

The selection of the appropriate and relevant method is of crucial importance when conducting research on endocrine disrupting chemicals (AWWA RF/Global Water Research Coalition (GWRC), 2005). Therefore, the need exists to develop a recommendation for a suite of suitable and reliable methods available for conducting this analysis.

10. The EDC toolbox project of GWRC

Endocrine disruption is an important research area for GWRC members and a number of joint research efforts have been undertaken. The project "Tools to Detect Oestrogenic Activity in Environmental Waters" (the EDC Toolbox) was jointly funded by the GWRC members AWWARF, UKWIR, WERF, and WSAA, and carried out by a project consortium of CRC WQT, KIWA, TZW, and WRC. WERF was the lead agent of this joint effort and CRC WQT was the coordinator of the consortium.

Additional projects, the GWRC toolbox project "In vitro bio-assays to detect oestrogenic activity in environmental waters" (2006) and WRC 1816 ("The Compilation of a Toolbox of Bio-assays for Detection of Oestrogenic Activity in Water", (in press) are projects that have looked at the various options available to recommend the most suitable test methods for assessing oestrogenic activity in water and includes both *in vitro* and *in vivo* bio assays. The *in vitro* tests can be broadly classified as either reporter gene assays or cellular proliferation assays.

The toolbox projects' results of bio-assays are important for this study as they describe the assays available together with their advantages and disadvantages.

The following table (Table 4) specifies the various assays and the type of assays investigated in the jointly funded toolbox projects. The project was jointly funded by members of the Global Water Research Coalition (GWRC), and evaluated a selected set of bio-assays, including yeast oestrogen screen (YES), ER-CALUX, MELN, T47D-KBluc and E-Screen assays. Tap water, spiked with known oestrogenic chemicals such as hormones, alkylphenols, phthalates, pesticides and phyto-esterols, and environmental samples from sewage, river, groundwater and treated drinking water were tested.

Table 4: Description of some *in vitro* and *in vivo* tests available for oestrogenic activity measurement

***In Vitro* Assays**	**Description**	**Specific Assay**
Reporter Gene Assays	Naturally oestrogen-responsive reporter gene-assays	Alkaline phosphatise (ALP) Ishikawa cell-line (human endometrial carcinoma
	Recombinant Yeast Assays	Recombinant Yeast Screen (YES), Yeast-based reporter gene assay Yeast two-hybrid assay Hybrid receptor yeast-based assay yEGFP Bio-assay
	Recombinant Mammalian Cell Reporter gene assay	T47D-KBluc reporter gene assay MVLN reporter gene bio-assay
Cell proliferation Assay	MCF-7 or T47-D human breast cancer cells	E-screen
***In Vivo* Assays**	**Description**	**Specific Assay**
	Vitellogenin induction	Catfish VTG Fish Vitellogenin
	Gonadal sex ratio and intersex	-

These studies showed that some techniques are sufficiently advanced and can be used as a cost-effective first-pass detection system. These methods need to be carried out in conjunction with complementary standard analytical methods to measure oestrogenic pollutants in environmental waters. However, a major short coming is the lack of standardization for data analysis or interpretation. This was identified as a crucial step forward towards accurate bio-assay-derived oestrogenicity measurements.

The studies looked at the various bio-assays and their reproducibility, robustness, inter-laboratory variability and their ability to integrate into a regulatory framework. Figure 5 and Figure 6 illustrate the results from these studies.

The results show that the ER-CALUX and E-Screen assays successfully detected oestrogenicity in environmental water samples even at very low levels of oestrogenicity (from 0.1 to 320 ng/ℓ EEQ) (GWRC 2008). The oestrogenic activity measured in these bio-assays was very similar to that predicted based on comprehensive chemical analysis using the various corresponding methods. The results indicate that both ER-CALUX and E-Screen can be used to screen oestrogenicity in environmental water samples. The T47D-K-Bluc assay was very similar to the ER-CALUX, but these conclusions were based on a more limited dataset, and should be considered critically.

The MELN assay tested gave good qualitative data, identifying low and high oestrogenic activity in the samples. However, accurate quantification was more problematic, possibly due to matrix interference from complex matrices (such as sewage) in this assay.

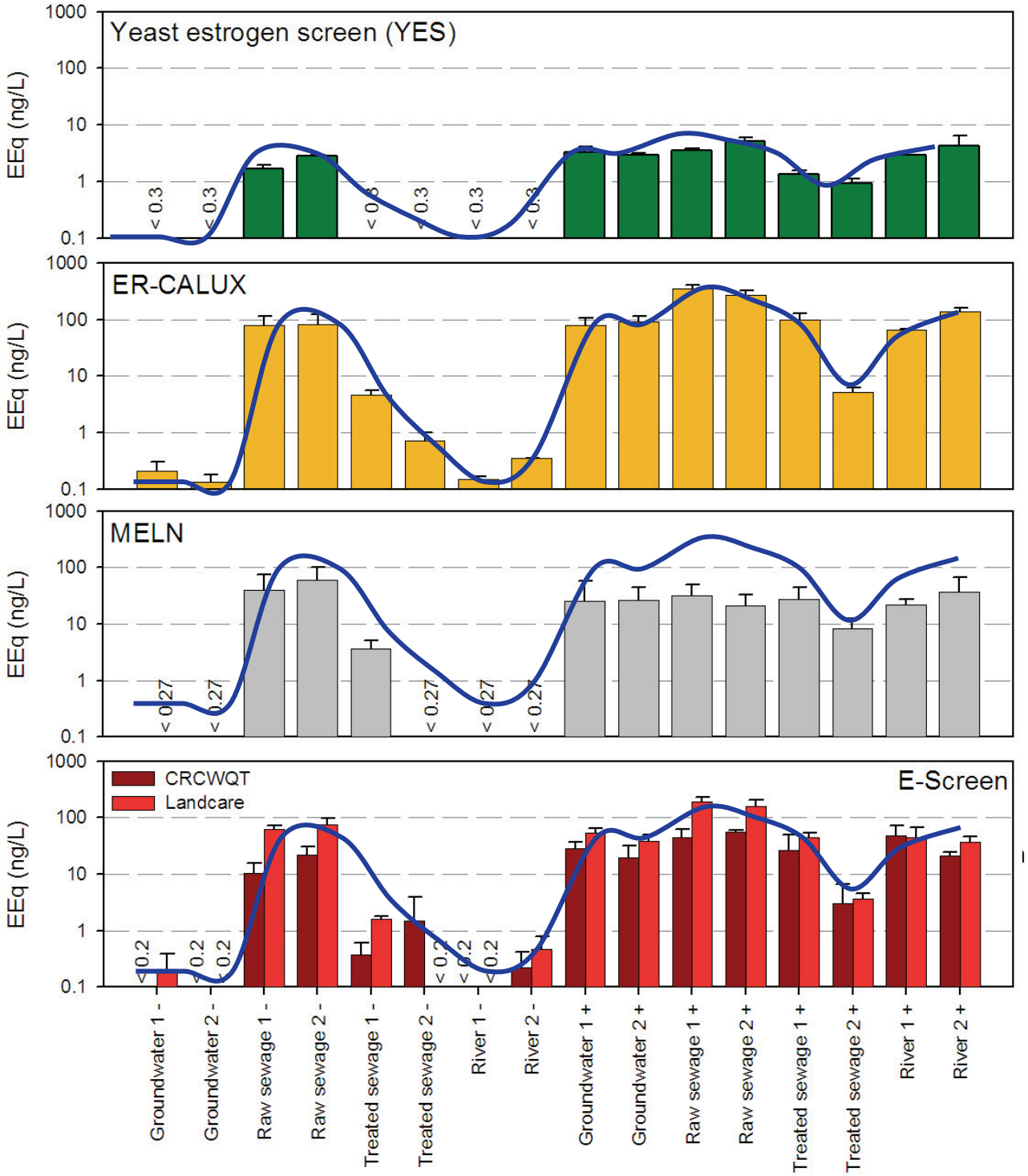

Figure 5: Variability between bio-assays in environmental samples (spiked samples on right hand side and un-spiked on left side) Source: GWRC, 2008; De Jager, 2008.

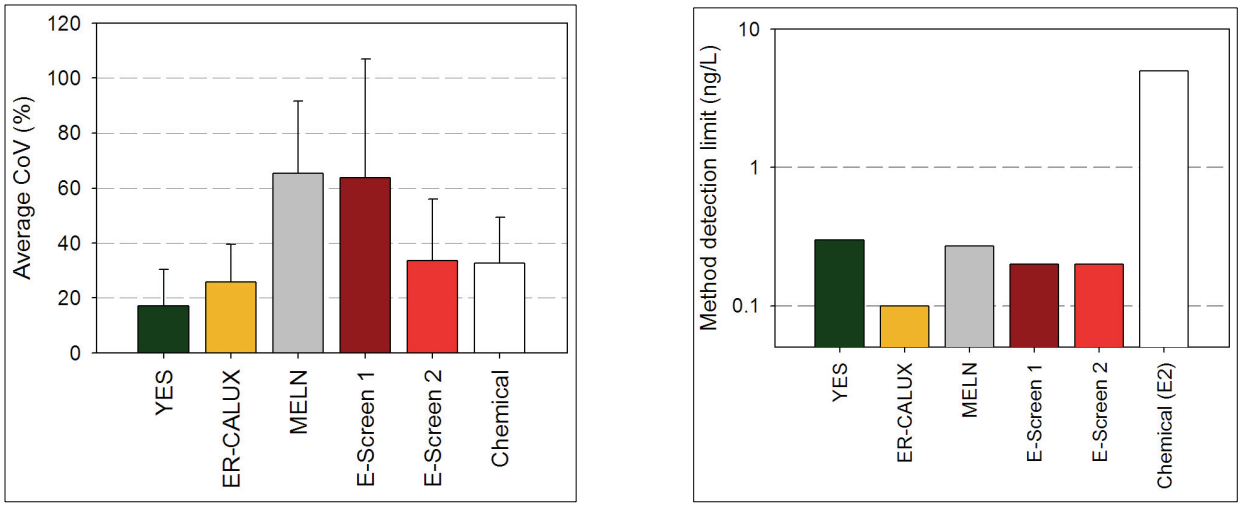

Figure 6: Variability – coefficient of variation between bio-assays with environmental samples and sensitivity (detection limits) of tests. Source: De Jager, 2008; GWRC, 2008.

There are many shortcomings with regards to these bio-assays, namely

- Cost: Running costs were in the order of R 2000 per sample at 2009 prices.
- Time: The tests were quite labour intensive. At present a laboratory with one technician is only able to process 4 samples per week. This severely limits recommending routine testing.
- Capacity: Capacity to perform these tests in South Africa is limited. There are only a few experienced and trained individuals capable of carrying out these tests as well as few laboratories available with the necessary equipment to conduct the bio-assays. At the time of writing, there were 2 active laboratories at universities set up to conduct these bio-assays on a routine basis. Other universities and water laboratories may be capable of carrying out the test with early notification and allowing them enough time to set-up the experiments / laboratory for such analyses.
- One of the problems in using *in vitro* assays to analyse environmental water samples is the presence of cytotoxic compounds.

Standard operating procedures would be required for sample collection, transport, and the SOPs for the testing method. More importantly also the SOP for interpretation of the results and this has not been achieved international or standardised yet.

EEQs are calculated in different ways depending on the laboratory doing the analyses. This is an issue that will need to be standardised, and has been recognised by the WRC, University of Pretoria and US EPA. The method used to calculate EEQs measured the mean maximum response of the positive control which was then set as 100% and all other samples were

expressed relative to this maximum response in the same assay. This method to calculate EEQs was preferred to other methods in which the relation between the EC50 of the standard substance and the EC50 of the sample are reported as EEQ.

- Advances in methodologies have now been made with regard to testing for thyroid activity (OECD, 2006). Tests are close to being standardised, similar to the process that oestrogen activity bio-assays went through in the GWRC study (2008). Generally, the amphibian metamorphosis assay has potential for detection of thyroid disrupters, reflected in discussions towards the OECD establishing future test guidelines for endocrine disruption in amphibians. However, further research is required in order to explore the potential of such an assay. During metamorphosis in amphibians, certain tissues are resorbed, or created to form an adult organism capable of surviving in a different habitat. Thyroid axis control of the metamorphosis in amphibians is highly complex but two principles remain constant, 1) metamorphic events are triggered by TH (thyroid hormone), and 2) tissue responsiveness to TH is based on selective response based on the hormone interaction with the receptor (OECD, 2006).

11. What to do if the trigger value is exceeded

Figure 7 depicts possible steps to take when the trigger value is exceeded in treated drinking water samples.

- If the trigger value of 0.7 ng EEQ/ℓ is exceeded in the screening test it is recommended that a more detailed investigation be carried out.
- After insuring that all SOPs were adhered to, a repeat sample should be taken and analysed.
- If the trigger value is once again exceeded, a situational analysis needs to be conducted. This entails assembling a multidisciplinary team to assess where the possible sources or causes of the contamination arise from.
- An assessment of the drinking water treatment processes and operations should be conducted to ensure that all processes were functioning optimally. If all processes are in place and functioning optimally, a total catchment to tap assessment should be conducted as described by the WHO (2004) and most municipalities in South Africa.
- Targeted chemical analysis should then be done to assess which chemical or group of chemicals are responsible for the oestrogenic activity.
- It is recommended that routine monitoring for oestrogen activity continue.

Although a trigger value is proposed, this does not replace a guideline value. A guideline value can only be proposed once more scientific evidence exists.

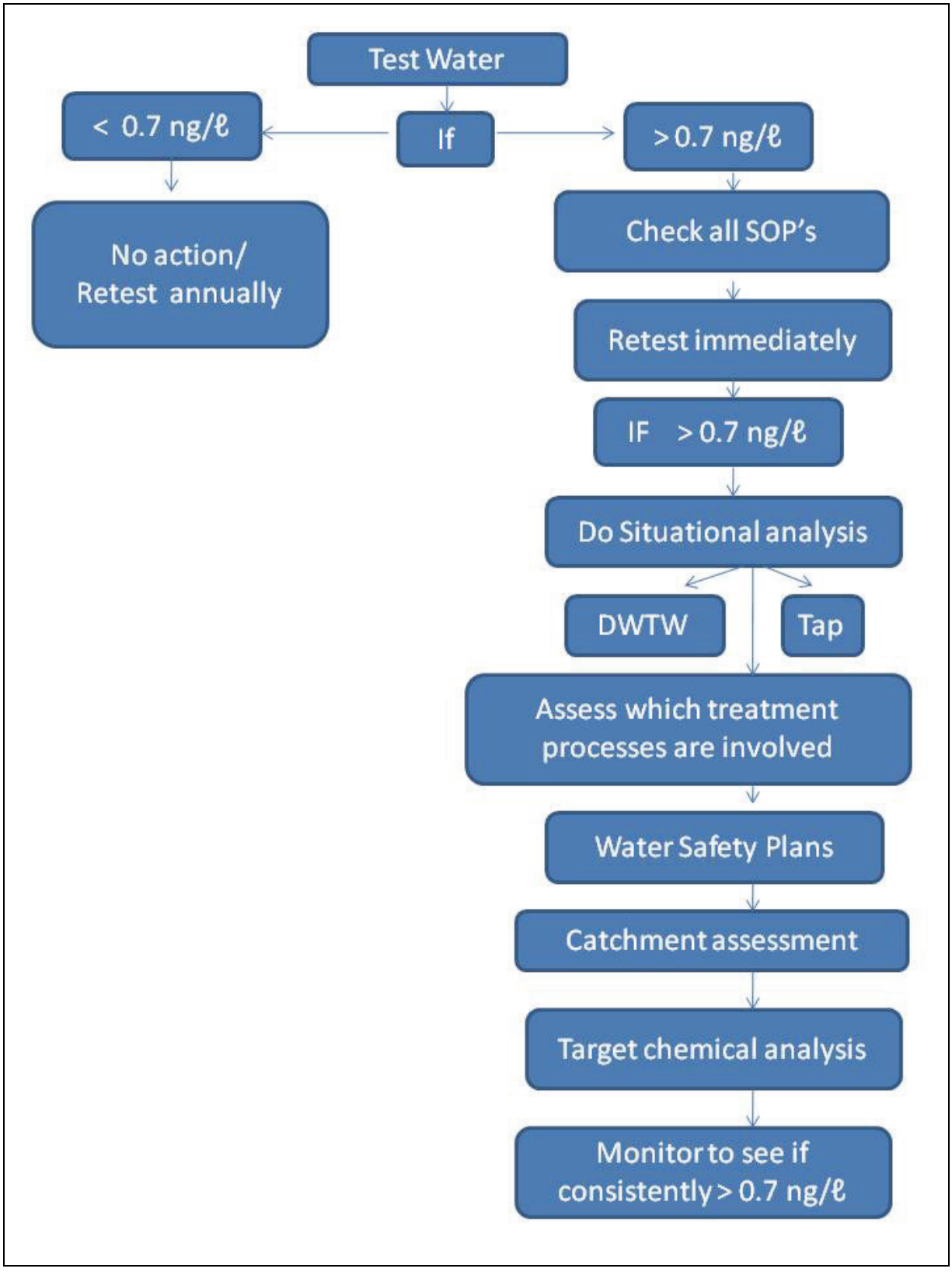

Figure 7: Procedure if trigger value exceeded

12. Drinking water treatment processes and their removal efficiencies of endocrine disruptors

Most drinking water treatment facilities will not be in a position to test for oestrogenic or other endocrine activity due to either lack of capacity or for financial reasons. What has been observed in the previous sections is that of the waters tested, most drinking waters appear to contain less than the recommended trigger value for oestrogenic activity and would not require specific additional investigation.

In general, studies have shown that each of the individual steps in the drinking water treatment process removes oestrogenic activity to some degree (Kirk et al., 2001; US EPA, 2001; Snyder et al., 2003).

Conventional water treatment refers to the treatment of water from a surface water source by a series of processes aimed at removing suspended and colloidal material from the water, disinfecting the water and stabilising the water quality. Conventional treatment of water for domestic use involves a number of treatment steps, namely, coagulation, flocculation, sedimentation or flotation and sand filtration, ending with disinfection. Advanced water treatment processes are typically used if water contains high levels of dissolved solids. The additional treatment processes (Figure 8 (WRC, 2002)) include pre-treatment, with further treatment steps including reverse osmosis, and activated carbon treatment. .

Internationally many studies have looked at the removal of chemicals and endocrine disrupting activities in drinking water and wastewater treatment processes. It has been shown that these compounds are not entirely removed and that additional advanced treatment processes are often needed (Kirk et al., 2001; US EPA, 2001; Snyder et al., 2003).

The studies illustrating the removal of these compounds have been summarised, and laboratory or bench scale studies included with environmental samples (Snyder et al. 2003). When this has not been available then predictions based on results with chemicals with similar qualities has been used. The following table from Snyder et al (2003) provides a summary of the processes and their removal efficiencies of endocrine disrupting chemicals (Table 5).

The most effective removal results from activated carbon, ultraviolet irradiation, reverse osmosis and degradation. Degradation includes bio- and photo-degradation and activated sludge treatment. Independently, the US-EPA (2001) evaluated the various components of the drinking water treatment process for their removal of specific endocrine disruptors. Granular activated carbon was identified as the most efficient method to be used for the removal of EDCs from drinking water. The EPA document presents treatment processes to remove EDCs from drinking water.

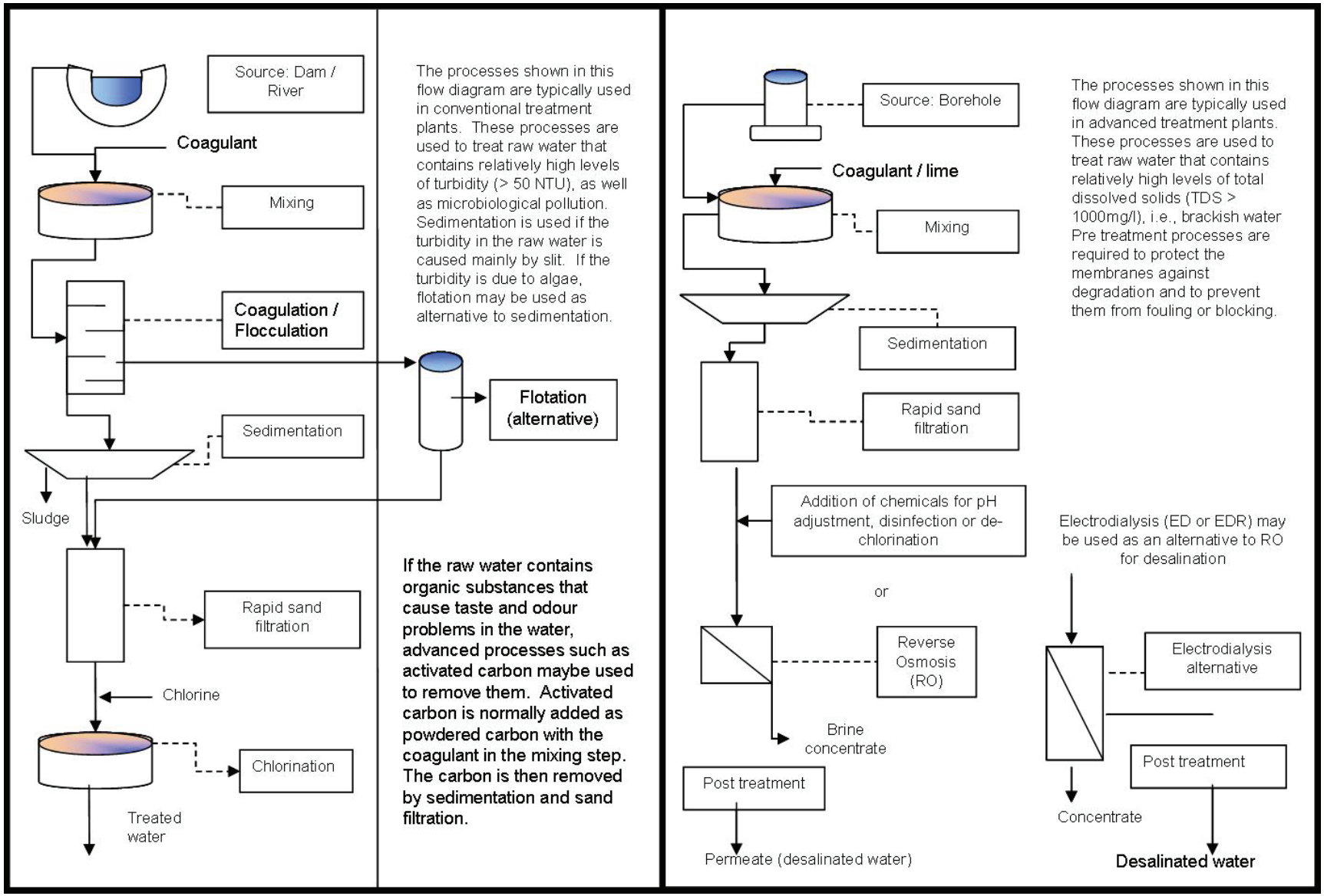

Figure 8: Conventional Water Treatment (left) and Advanced Water Treatment Facilities (right) (Source: WRC, 2002)

Table 5: Removal of ED chemicals in water treatment processes (source Snyder, 2003)

Type of process	Activated carbon	Biological activated carbon	Ozone (O_3)	UV	Cl_2 / ClO_2	Coagulation / Flocculation	Softening	Nano-filtration	Reverse osmosis	Degradation
Pesticides	**E**	**E**	L-E	**E**	P-E	P	**G**	**G**	**E**	**E**
Industrial chemicals	**E**	**E**	F-G	**E**	P	P-L	P-L	**E**	**E**	**G-E**
Steroids	**E**	**E**	**E**	**E**	**E**	P	P-L	**G**	**E**	L-E
Metals	**G**	**G**	P	P	P	F-G	F-G	**G**	**E**	P, E
Inorganics	P-L	F	P	P	P	P	**G**	**G**	**E**	P-L
Organometallics	**G-E**	**G-E**	L-E	F-G	P-F	P-L	P-L	**G-E**	**E**	L-E
L low 20-40% F fair 40-70% P Poor <20% G Good 70-90% E Excellent >90%										

One can therefore expect that a large proportion if not all of the endocrine disrupting chemicals will be removed in a properly functioning drinking water treatment works. If this is not achieved, more advanced processes can be used to achieve greater removal efficiency.

The above information should be used to qualitatively assess the water treatment facilities in South Africa based on local conditions. Information is available on every drinking water and waste water treatment facility in the country via e-WISA's website. The website information includes a classification of the treatment facility based on the size of the treatment works. In addition, it shows the maximum capacity of each of these treatment works.

Figure 9 depicts a screen shot / print screen from the website indicating the treatment facilities for the Western Cape Province.

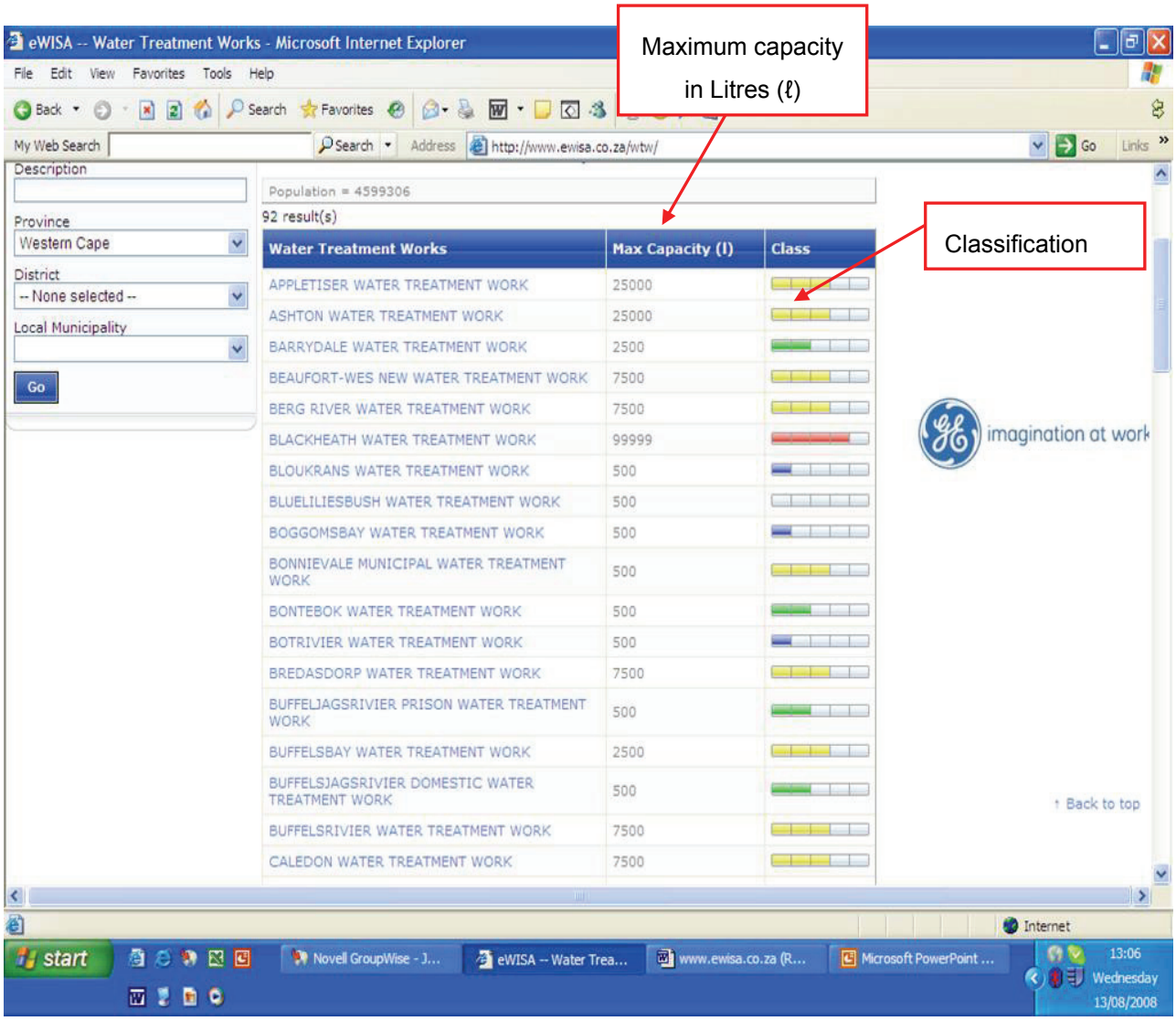

Figure 9: Classification and capacity of Western Cape Water Treatment Facilities (eWISA.co.za, 2009)

Although the available information is rather valuable, the classification however does not give any indication of the treatment processes at each of these treatment works or whether the treatment facility is functioning within its capacity (in other words whether it is working at all and whether it is functioning within its design criteria/ specifications).

It is recommended that, as a way forward this information should be collected. In addition, the local treatment facility conditions in terms of size, functioning, and type of treatment processes involved at each of these treatment facilities should be collected. Local conditions should then be compared to the Snyder (2003) report to assess whether our treatment facilities are capable of removing endocrine disrupting activity from our waters. This might be a more feasible but also a more cost effective approach than to have all treatment facilities undergo testing of their waters. In addition, this information would also be valuable for a wider population since other projects often

require information regarding the treatment processes available at the different water treatment facilities.

In addition to the above information, information regarding the different agricultural activities to qualitatively assess the potential for pesticide use and other endocrine disrupting chemicals as well as the different industries in South Africa should be listed and mapped. GIS interactive electronic maps of this information could then be designed to make the end-product more user-friendly.

- This approach is comparable to a qualitative risk assessment on the potential human health risks from industry and agriculture where treatment facilities would not be able to effectively remove these substances from the water, potentially posing human health risks. This could support water safety plans (recommended by WHO and adopted by DWAF) that are currently being implemented by municipalities in the terms of the blue-drop initiative. A simple map could be produced to illustrate oestrogen activity of South Africa's water using a simple screening test to screen the water treatment works throughout the country for their efficiencies in the removal of hormones and the results mapped.

13. Conclusions

The current project achieved its objectives by addressing each of the four aims. The trigger value approach suggested as the framework to deal with endocrine disrupting activity in drinking water in South Africa was tested and found feasible. Not only did it work for treated municipal supplies, but also for environmental waters from rivers and streams which are at times used for drinking purposes.

It is still recommended that a battery of *in vitro* and *in vivo* bio-assays be used to assess for endocrine disrupting activity. It is suggested, that water be screened for endocrine disrupting activity and compared to the trigger value. If below the trigger value, no action needs to be taken. If however, the activity exceeds that of the trigger value, further analysis and investigation is recommended. A tiered approach is therefore recommended. Much research is however still required in terms of developing a set of bio-assays to assess water for endocrine disrupting chemicals. *In vivo* tests will need to be included to be able to contend with the issue of trans-generational effects. Researchers should also focus on standardising procedures and validate them. Capacity should be extended and research should aim to identify cheaper and less time consuming processes in the long run.

Thyroid activity testing is far enough advanced to recommend. The Organisation for Economic Co-operation and Development, or OECD (2006) has recently validated the thyroid activity test. More research is however needed; also to include thyroid, reproductive, neurological and immunological activity.

14. Recommendations for Future Research

Most drinking water treatment facilities will not be in a position to test for oestrogenic or other endocrine activity due to either lack of capacity or for financial reasons. From the results, it is clear that of the waters tested, most drinking waters appear to contain less than the recommended trigger value for oestrogenic activity and would not require specific additional investigation. One can therefore expect that a large proportion if not all of the endocrine disrupting chemicals will be removed in a properly functioning drinking water treatment works. If this is not achieved, more advanced processes can be used to achieve greater removal efficiency.

- It is recommended that, as a way forward, information regarding the local conditions should be collected in terms of size, functioning, and type of treatment processes involved at each of these treatment facilities.
- Local conditions should then be compared to the Snyder (2003) report to assess whether our treatment facilities are capable of removing endocrine disrupting activity from our waters. This might be a more feasible approach than to have all treatment facilities undergo testing of their waters and it would also be more cost-effective.
- In addition to the above information, information regarding the different agricultural activities to qualitatively assess the potential for pesticide use and other endocrine disrupting chemicals as well as the different industries in South Africa should be listed and mapped.
- GIS interactive electronic maps of this information could then be designed to make the effort more user-friendly.
- As a first screen, a simple screening assay (using GC-MS) for a standard hormone such as oestrone could be used to screen the water treatment works throughout the country for their efficiencies in the removal of hormones.

This is comparable to a qualitative risk assessment on the potential human health risks from industry and agriculture where treatment facilities would not be able to effectively remove these substances from the water, potentially posing human health risks. This could support water safety plans, as recommended by WHO, that currently need to be implemented by each municipality.

15. Capacity Development

The original capacity development planned for the project could not take place as the student at the University of KwaZulu-Natal left the university towards the middle of the project due to health reasons. Capacity development took place with one female researcher, Maronel Steyn, being

exposed to the process of guideline development within the risk assessment process used within the guideline development process and understanding the endocrine system.

16. Archiving of Data

No data with the exception of that given in the report was created in the execution of this project.

17. Conference Presentation and Proceedings

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