Health Risk Assessment Protocol for Endocrine Disrupting Chemicals

Report to the Water Research Commission

by

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WRC Project No KV 206/08
ISBN 978-1-77005-686-2

March 2008

The publication of this report emanates from a project entitled *Health risk assessment protocol for endocrine disrupting chemicals (EDCS) in water* **(**WRC project number K8/648).

Executive Summary

Background

Various chemicals have shown endocrine disrupting properties, causing adverse effects in animals ranging from developmental and reproductive effects to structural deformities, cancer and immune system deficits. Numerous wildlife species, including mammals, birds, fish, reptiles, and molluscs have already been affected. It is therefore anticipated that endocrine disrupting chemicals affect humans, and there is some evidence of these effects on human health. However, there is an ongoing debate about this.

Of particular importance in this report, are adverse human health outcomes relating to chemical constituents that elicit endocrine disrupting properties and are potentially present in drinking water. Preventative measures need to be put in place to minimise exposure of people to endocrine disrupting chemicals in water that would elicit adverse health effects. One such precautionary measure would be the derivation of guideline values for endocrine disrupting chemicals in drinking water. Drinking Water Quality Guidelines provide safe levels for microbiological, physical, as well as chemical properties of water to ensure that the water people drink are safe and that exposure to this water would not cause any adverse health effects, even if used for a lifetime. Guidelines also provide water treatment operators with targets for treatment to provide safe drinking water.

The main objective of this report was to provide an initial point for a framework for developing guidelines for South Africa for endocrine disrupting chemicals in water used for domestic purposes. This report discusses the general and current understanding of chemicals with endocrine disrupting properties in water and provides a summary of the literature available to us. International management of endocrine disrupting chemicals are discussed, and finally a method is recommended for South Africa.

Methodology

Available methods used internationally to deal with setting guidelines for endocrine disruptors in drinking water were assessed. These methods were scrutinised to develop an understanding of their implications to be able to recommend a framework for use in South Africa. A description of available methods to set guideline values for potential harmful chemicals was given, in addition to a short description of available methods to determine potential health risks associated with endocrine disruptors. The report dealt with current health risk assessment processes and how it can be used to develop guideline values for chemicals in water. Finally the uncertainties or drawbacks in terms of endocrine functioning are discussed.

Results and Recommendations for Future Research

The suggested or proposed framework for endocrine disrupting chemical guidelines for South Africa is based on a first level screening test for reproductive endocrine disrupting capability rather than for individual chemical concentrations. Should endocrine disruption be detected, then a more detailed assessment is recommended. The screening test should use a battery of *in vitro* and *in vivo* tests quantitatively expressing the results of endocrine activity of a water sample containing a mixture of chemicals in terms of their relative potency is recommended. It is recommended that an approach similar to toxic equivalency factors be used for hormones and their activity in water and expressed in terms of estrogen equivalency factors. A value above which a more detailed assessment is recommended would be the “trigger value”. This trigger value might be 0.5 ug/L estrogen equivalents – based on a formula for water quality guideline calculations described in more detail in the main body of the report.

It is acknowledged that the proposed approach described in this document has severe

shortcomings. We are (as is the rest of the world) trying to propose a method to protect human health. A start must be made with regards to controlling possible endocrine disrupting chemicals in our water. We will need to reassess the approach as new methods and more data becomes available. The problem of developing guidelines for endocrine disruptors in water is not unique to South Africa. The Global Water Research Coalition (GWRC) selected Endocrine Disrupting Compounds as the first item of research cooperation, with research projects about the occurrence and fate of endocrine disrupting chemicals during wastewater treatment and sewage and soil in progress.

In summary, endocrine disrupting chemicals occur in South African waters and we need to respond to this without waiting for perfect data. At this stage we are not certain at which levels endocrine disruptors adversely affect health. In addition it is not practical to test for each individual chemical that may cause endocrine disruption. We therefore need to make use of methods that test for endocrine disrupting activity.

It is recommended that a battery of tests eventually be included, and that these tests include a number of taxa. We will also need to include endocrine effects other than reproduction such as thyroid and immune effects. One of the more difficult problems we will need to address is the trans-generational effects of endocrine disruptors. Research on this problem will continue and new values will become available as our understanding and information improves. It is recommended that this proposed framework be tested for its feasibility within the domestic water quality arena. This recommendation has been put into place through WRC project K5/1749: Development and testing of a health risk assessment framework to derive guidelines for endocrine disruptors (EDCs) in drinking water.

Capacity Development

Capacity development took place in the form of one female researcher, Maronel Steyn, being exposed to the process of guideline development, the risk assessment process used within the guideline development process and understanding the endocrine system.

Archiving of Data

No field or experimental data was collected for the study as the study assessed the available literature to recommend an approach for guidelines for endocrine disruptors. These recommendations are being taken forward in WRC project K5/1749.

Conference Presentation and Proceedings

Genthe, B and Steyn, M. (2006) An overview of health effects of endocrine disrupting chemicals in water – where are we in South Africa? WISA, Durban, 21-26 May.

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1. BACKGROUND TO THE PROJECT

Various chemicals have shown endocrine disrupting properties, causing adverse effects in animals ranging from developmental and reproductive effects to structural deformities, cancer and immune system deficits. Numerous wildlife species, including mammals, birds, fish, reptiles, and molluscs have already been affected. It is therefore anticipated that endocrine disrupting chemicals affect humans, and there is some evidence of these effects on human health (Solomon and Schettler, 2000; WHO/IPCS, 2002). However, there is an ongoing debate about this (Breihaupt, 2004; Safe, 2004).

Of particular importance in this report, are adverse human health outcomes relating to chemical constituents that elicit endocrine disrupting properties and are potentially present in drinking water. Preventative measures should therefore be put in place to avoid / minimise exposure of people to endocrine disrupting chemicals in water that would elicit adverse health effects.

One such precautionary measure would be the derivation of guideline values for endocrine disrupting chemicals in drinking water. Drinking Water Quality Guidelines provide safe levels for microbiological, physical, as well as chemical properties of water to ensure that the water people drink are safe and that exposure to this water would not cause any adverse health effects, even if used for a lifetime. Guidelines also provide water treatment operators with targets for treatment to provide safe drinking water.

The main reason for this report is to provide a starting point in the form of a framework for developing guidelines for South Africa for endocrine disrupting chemicals in water used for domestic purposes. This report discusses the general and current understanding of chemicals with endocrine disrupting properties in water and provides a summary of the literature available to us. International management of endocrine disrupting chemicals is discussed. For decades, the regulatory approach in South Africa in terms of water quality management and guideline development was to prevent hazardous substances above certain concentrations from entering the environment. This approach mainly focuses on single substances and their risk to human health. This is also currently the approach followed by the Human Health Risk Assessment process.

People are seldom (if ever) exposed to a single hazardous substance. From literature, it is evident that mixtures of chemicals have different effects than the single chemicals evaluated on their own, and thus a major limitation in the approach so far. DWAF in 2003 changed this approach by adopting the whole effluent toxicity approach from the Netherlands and implementing the National Toxicity Monitoring Programme. This approach of evaluating mixtures of chemicals instead of single substances is also recommended in this framework.

Note: Although the framework addresses endocrine disrupting chemicals in general, more attention is given towards reproductive health effects at this early phase of the framework, since more research have been done in this field.

2. INTRODUCTION

Endocrine disruption and the effects of endocrine disrupting chemicals is such a controversial subject that as Krimsky (1998) stated, “We’re dealing with a mechanism that is so complex that there isn’t even consensus on the definition yet”. Some refer to it as endocrine modulators, some to endocrine active substances and others prefer to call them endocrine disruptors.

In 1996, Kavlock and colleagues defined an endocrine disrupting chemical as:

“An exogenous agent that interferes with the production, release, transport, metabolism, binding,

action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes."

Since *interfering* with the production, transport, etc. within the human body does not necessarily mean that it causes adverse health effects, the WHO instead used a slight modification of the Weybridge (1996) definition to define these chemicals as follows: “An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations”.

Of the 80 000 chemicals that are registered for commercial use we need to establish how many have endocrine disrupting properties. A number of chemicals are suspected of having these endocrine disrupting effects. General classes of chemicals considered to be endocrine disrupting include insecticides, herbicides, fumigants and fungicides used in agriculture as well as in the home, detergents, resins, and plasticizers.

Various naturally occurring as well as synthetic chemicals have been identified that elicit endocrine activity. The Centres for Disease Control and Prevention (CDC) have classified 48 chemicals as endocrine disruptors (Choi et al., 2004), whereas the Japanese have listed 67 chemicals as suspected endocrine disruptors (Tohyama et al., 2000).

Chemicals considered to be endocrine disruptors have been found in South African waters and wastewaters in many previous studies (Bornman et al., 2005; Slabbert et al., 2005; Aneck-Hahn et al., 2002; Dalvie et al., 2003). The DWAF came up with a priority list of suspected endocrine disrupting chemicals for South Africa. Thirty-three (33) substances were listed as potential endocrine disrupting chemicals that are frequently used in South Africa and occur in different water bodies around the country.

There is still so much not understood about endocrine disrupting chemicals and the extent of their impact due to a lack of information in several key areas. Most of the current evidence involves adverse health effects in wildlife that have been successfully linked with exposure to endocrine disrupting chemicals.

From this evidence it can be assumed that exposure to endocrine disrupting chemicals is likely in humans, and that preventative action needs to be taken. It is clear that the use of these chemicals needs to be managed and exposure prevented or limited. However, for economic reasons, scientific evidence of adverse effects is needed before a chemical compound can be defined as an endocrine disruptor, and therefore be controlled.

One of the main questions facing scientists and policy-makers is: When is there enough scientific understanding to proceed with actions? We are therefore caught between many urgent calls for action and the realisation that the means and knowledge to achieve these actions are only inadequately understood. Internationally, this has inevitably led to the adoption of simplified models with which to devise assays and hazard definition /risk assessment methodologies. While accepting that this involves necessary compromises, it is important not to forget that these compromises have been made, and to remain open to the impacts that new insights and understanding will have on these simplified yet enabling models (Miyamoto and Burger, 2003).

As a precautionary approach, action called for a proposed framework for dealing with these chemicals suspected of eliciting endocrine disrupting properties present in drinking water in South Africa. The focus of this framework is endocrine disrupting chemicals in water used for domestic purposes, what their sources are, how they function, how the endocrine system functions and how it becomes disrupted, the concentration of these chemicals in the environment, how we can interpret these concentrations, the need for guidelines for the chemicals in the environment, and how we can work towards that.

3. NORMAL FUNCTIONING OF THE ENDOCRINE SYSTEM

The endocrine system provides the key communication and control link between the nervous system and bodily functions such as reproduction, immunity, energy control, metabolism and behaviour (e.g. fight or flight response), and growth and development.

The endocrine system is made up of three components, namely

- endocrine glands,
- hormones and
- receptors

The endocrine glands secrete hormones, the hormones circulate around the body via the blood stream and modulate cellular or organ functions by binding with receptors in the target cells. Lastly the receptors in the target cells, once activated by binding of the hormone, regulate the functions and processes in the tissue through interactions with the cell's DNA or other complex intracellular signalling processes. The target cells of the endocrine system can be so sensitive, that the blood plasma level needed for a response may be at a concentration as low as 1 picoMole or 10^{-12} M (Water Research Centre, 2005). There are at least eight endocrine glands in mammals that produce over 40 hormones (WRC, 2005).

Table 1 summarises the major glands and hormones involved in the endocrine system.

Table 1: The endocrine systems' glands, hormones and their function

Gland	Hormones	Functions
Hypothalamus	Releasing hormones	Stimulate pituitary activity
Pituitary	Trophic (stimulating) hormones	Stimulate thyroid, adrenal, gonadal and pancreatic activity
Thyroid	Thyroid hormones	Regulate metabolism, growth and development, behaviour and puberty
Adrenal	Corticosteroid hormones Catecholamines	Regulate metabolism Regulate behaviour
Pancreas	Insulin and glucagons	Regulate blood sugar levels
Gonads	Sex steroid hormones (androgens and oestrogens)	Regulate development & growth, reproduction, immunity, onset of puberty and behaviour

The endocrine system regulates processes as diverse as blood pressure, smooth muscle contraction, fluid balance and bone-resorption (IPCS, 2002). For many of the systems the setup is programmed during foetal development and an abnormal environment during this critical stage can result in permanent mis-programming (IPCS, 2002).

A similar, but not identical, endocrine system to that of humans is found in nearly all vertebrates including other mammals, fish, amphibians, reptiles and birds, although the precise structures and roles of the various organs and hormones differ between different groups (EUROPA EU, 2004). Invertebrates also have endocrine systems that control a similar range of body functions although these have evolved along markedly different lines to those of vertebrates (EUROPA EU, 2004, WHO/IPCS, 2002).

4. WHAT IS ENDOCRINE DISRUPTION?

According to the Oxford Dictionary (2006) the word “disrupt” means to interrupt or disturb an activity or process. Endocrine disrupting or active chemicals interrupt or disturb an activity or process within a living organism. Since the endocrine system is responsible for a vast majority of different functions as well as handling homeostasis in the human body, any interruption or disturbance of the processes may cause adverse functioning of various bodily systems (e.g. growth and development).

Endocrine modulators or endocrine disruptors may be natural products or synthetic chemicals capable of direct interaction with oestrogen receptors, other hormones, or transcription factors in the biochemical pathway of hormone activity.

The largest group of known endocrine disruptors are the chemicals that act as hormone mimics via estrogen receptor mechanisms (Welshon et al., 2003). The naturally occurring oestrogens in humans include 17β - oestradiol, oestrone, and oestriol. Oestradiol is the most potent natural oestrogen targeting the reproductive tract, bone synthesis, and affecting fat distribution throughout the body (Kroes et al., 2000).

These environmental oestrogens can enhance (an agonist) or inhibit (an antagonist) the action of endogenous hormones. In some instances, this group of substances can act as both an agonist and an antagonist, depending on the target tissue (Kroes et al., 2000). Cross-talk may occur between different systems and may result in systems other than the anticipated one being affected. It is therefore very important to be cautious in extrapolating *in vitro* hormone activity detected to the *in vivo* situation (WHO, 2002). The cross-talk phenomenon means that even when an environmental chemical is shown to have a steroidal hormone activity, it may also possess other relevant activities. For example, some endocrine disruptors such as DDT isomers and certain phthalates (plasticizers used in industry) may have both anti-androgenic as well as oestrogenic effects.

The normal functioning of the endocrine system can be disrupted in three different ways (Kimball, 1994; Australian Academy of Science, 1998):

1 **Mimics** - By mimicking a natural hormone and locking onto a receptor within the cell, the disrupting agent may give a signal stronger than the natural hormone or a signal that occurs at the “wrong” time.

2 **Triggers** - The disrupting substance can bind to a receptor within a cell, thereby preventing the correct hormone from binding. The normal communication and signal will fail to occur and the body will therefore fail to respond properly.

3 **Blockers** - The disruptors can interfere or block the way natural hormones and receptors are made or controlled. This interference or blockage may occur only if relatively large doses of the substance are present.

5. WHAT TYPE OF ADVERSE EFFECTS CAN BE EXPECTED FROM ENDOCRINE DISRUPTORS?

The effects of Endocrine disrupting chemicals may be either reversible or irreversible, immediate (acute) or delayed (chronic) and depend on the following factors (American Chemical Society, 1998):

- The type of chemical
- The kind of tissue exposed
- The dose, timing and duration of exposure
- Metabolism and elimination from the body

Level of toxicity

At least four major categories of adverse biological effects may be linked to exposure to endocrine disruptors, namely:

- cancer,
- reproductive and developmental alterations,
- neurological effects and
- immunological effects.

Endocrine systems that may be involved include the thyroid, adrenal, pituitary, and gonadal system. This includes cognitive effects, which have been observed in animals and humans (Schantz and Widholm, 2001).

The hypothesis that endocrine disruptors can cause cancer in humans is based largely on the clear association between exposure of females *in utero* to diethylstilbestrol (DES), a potent synthetic estrogen taken by pregnant women to avoid miscarriage, and subsequent onset of reproductive organ cancers (Silbergeld et al., 2002). In addition, it has been established that some other xenobiotics such as DDT, PCBs and tetrachloro t-dioxin are unequivocally carcinogenic (Tsuda et al., 2003).

The largest group of known endocrine disruptors are the chemicals that act as hormone mimics via estrogen receptor mechanisms (Welshon et al., 2003). Whether people that drink water containing low levels of these chemicals are at risk of adverse health effects is still debatable. One argument that is applied to support concern for low level exposure, is the strong affinity of hormonal substances for hormone sites even at such low levels. The *in vitro* estrogenic activity of estradiol (agonistic – EC25) is 0.003 nM (COMPARE, 2005).

Many synthetic chemicals suspected of causing adverse effects are persistent in the environment, tend to accumulate in fat tissue of humans and wildlife, and are released during times of stress, malnutrition, and pregnancy (American Chemical Society, 1998).

A concern regarding the bioaccumulation of certain chemicals has the potential for embryos and infants to be exposed at critical stages in their development through the womb or through their mothers' breast milk. The reproductive system and embryo are the two bodily systems that seem particularly vulnerable (American Chemical Society, 1998).

Based on this, the majority of the studies have focused on reproductive health effects. A number of adverse reproductive health effects have been observed in which endocrine disrupters could play a role, namely

- Declining sperm counts: Some studies in certain western countries have reported decreases in sperm numbers over the last 50 years. However, other studies in different regions have failed to detect such changes.

- Congenital malformations in children: In recent years there has been an increase in the incidence of hypospadias (a congenital abnormality of the urethra in the penis) and cryptorchidism (undescended testes) in humans. However, no causal association with chemical exposure has yet been established.

- Retarded sexual development: A few reports have been published suggesting that adolescents in polluted areas may take longer to reach puberty. However, the potential mode of action of any such effect is unknown.

Table 2 provides a list of the more common endocrine disruptors, their uses, mechanisms of action and health effects. Most of the effects listed below are reproductive health effects as most studies have focused on this endpoint.

All aspects of reproductive function are controlled by various endocrine communicating systems

that employ a large number of protein /peptide and steroid hormones, growth factors and other signalling molecules that affect target gene cell expression and or protein synthesis. The developing foetus may be more sensitive to the effects of exposure to environmental chemicals than the adult system. However, effects may not manifest until adulthood (WHO, 2003)!

The timing of exposure to endocrine disruptors is very important, with children and foetuses being the most sensitive. During foetal development different events occur during small "windows of time" and during this time the foetus will be very sensitive to hormone disruption (Jensen et al., 1995). For example, testis development occurs during early development in-utero with Sertoli cells (responsible for sperm production later in life) differentiating. Exposure to oestrogen at this time reduces the number of Sertoli cells (Jensen et al., 1995).

Table 2: Endocrine disruptors and their mechanisms of action (Source: Solomon & Schettler, 2000).

Chemical	Use	Mechanism	Health Effect
DES	Synthetic Oestrogen	Oestrogen receptor agonist	Humans (prenatal exposure):vaginal cancer, reproductive tract abnormalities (females); cryptorchidism, hypospadias, semen abnormalities (males)
Methoxychlor	Insecticide	Metabolite is an oestrogen receptor antagonist	Rodents: accelerated puberty, abnormal ovarian cycling (females);aggressive behaviour (males)
DDT	Insecticide	Metabolite(DDE) is an androgen receptor antagonist	Rodents (males): delayed puberty, reduced sex accessory gland size
Vinclozolin	Fungicide	Androgen receptor antagonist	Rodents (males): feminization, nipple development, hypospadias
PCBs	No longer manufactured; still in electrical transformers, capacitors, toxic waste sites, food chain	Accelerated T_4 metabolism, decreased T_4 levels, elevated TSH levels (high doses: thyromimetic)	Humans (in utero exposure): delayed neurological development; IQ deficits
Atrazine	Herbicide	Reduces gonadotropin-releasing hormone from hypothalamus, reduces pituitary LH levels, interferes with metabolism of estradiol, blocks oestrogen receptor binding	Rodents (females):mammary tumours, abnormal ovarian cycling. Humans: some evidence of breast and ovarian tumours
Dioxin	By-product of industrial processes including waste incineration; food containment	Aral hydrocarbon receptor agonist; increases oestrogen metabolism, decreases oestrogen mediated gene transcription, decrease oestrogen levels, decreases testosterone levels by interfering with HPG axis	Rodents (in utero exposure): delayed puberty, increased susceptibility to mammary cancer (females); decreased testosterone, hypospadiasis, hypospermia, delayed testicular descent, feminized sexual behaviour (males) Humans: decreased T_3 and T_4 levels decreased testosterone levels*cancer*

There is much debate about the significance of evidence that endocrine disruptors are affecting the health of the general population. Epidemiological studies are almost non-existent. However, surveillance of possible hormone related conditions show an increase in these conditions. Table 3 below provides an overview of the potential endocrine disrupting effects with regards to sexual development.

Table 3 Trends in human health effects potentially related to endocrine function

(Source: Solomon and Schettler, 2000)

End point	Trend	Degree of change
Hypospadias	Increasing incidence	3.3 - 4.3% per year
Cryptorchidism	Increasing incidence	1.6 - 3.5% per year
Sperm count	Decreasing	-5.3 to -0.7%/mL per year
Testicular cancer	Increasing incidence	2.1 – 5.2% per year
Prostate cancer	Increasing incidence	3 - 5.3% per year
Breast cancer	Increasing incidence	1.9 - 3.3% per year
Sex ratio	Shift toward females	-0.5 to -1.0 males/10 000 per year
Age at breast development	Shifting earlier	11.2-9.96 years in white US population

6. GLOBAL AND SOUTH AFRICAN SITUATION

Various environmental concentrations of endocrine disruptors have been detected and are reported on in the literature. For instance, in Holland 3.5 ug/L DEHP was found in water (ENDS, 1999). In Italy, 15-29 ng/L Bisphenol A and more than 1.2 ug/L nonylphenol were detected in a river (Lagana et al., 2004). Closer to home in the Western Cape, endosulfan was found in 32% of ground-waters, surface water and drinking water tested (Dalvie et al., 2003). The more contaminated site exceeded the European Drinking Water Standard of 0.1 ug/L, with an average of 3.16 +- 3.5 ug/L (Dalvie et al., 2003).

It is clear that endocrine disruptors are present in the environment at concentrations that may cause adverse health effects. In assessing the potential health risks it is necessary to establish the concentrations in the environment near the population of interest. Environmental exposure to chemicals may be via numerous routes and pathways. Exposure may be via the inhalation, dermal absorption or ingestion route, which can include ingestion of food and water.

In South Africa, the evidence of endocrine disruptors includes:

- levels of p-nonylphenol (p-NP) in drinking water and sources equal to those reported to cause feminisation in trout (Routledge et al., 1998),
- p-NP, polychlorinated biphenyls (PCB), DDT, DDE, heptachlor, endosulfan and the chlordanes in selected water and sediment samples (Bornman et al., 2005, Awofolu and

Fatoki, 2003)
- oestrogenic activity in water (Aneck-Hahn et al., 2002; Hurter et al., 2002)
- catfish collected from some sites had significant levels of selected endocrine disrupting chemicals in fat tissue (Barnhoorn et al., 2004).
- endosulfan was found in 32% of ground-waters, surface water and drinking water tested (Dalvie et al., 2003).
- A large number of surface water and effluent samples tested in the Gauteng Province were found to have significant estrogenic activity using the human yeast screen test (Slabbert et al., 2005).

7. HOW DO WE APPROACH / HANDLE THESE CHEMICALS?

Both natural and synthetic endocrine active chemicals are ubiquitous in the environment. Many chemicals are suspected of eliciting endocrine disrupting effects in animals and humans. Although a definite link cannot yet be scientifically proven between exposure to endocrine disrupting chemicals and human health, one needs to take preventative measures. Water authorities need to ensure that people are not exposed to unsafe drinking water. Safe treatment technologies and treatment levels need to be established to ensure human safety.

Banning or controlling of these chemicals may need to be initiated, but only once evidence has shown that there is an adverse effect of a large enough magnitude. Ongoing research in South Africa found endocrine activity in river waters in different areas of the country (Bornmann, et al., 2005; Barnhoorn, et al., 2004). However, due to all the uncertainties underlying this concept, we still do not know if the activity is enough to cause adverse health effects in humans. Other uncertainties include the working of these chemicals, synergistic effects, effects of mixtures of chemicals, environmental conditions, who is exposed, etc.

So far, countries have come up with priority lists of chemicals suspected of endocrine disrupting properties expected to elicit adverse health effects in humans and wildlife. Once the priority chemicals have been identified within a country and a particular drinking water supply, a management policy should be established and implemented to provide a framework for the prevention and reduction of these chemicals. Monitoring and water quality analyses programmes should be established to ensure that the quality of drinking water remains of good quality.

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.

Usually the studies used to derive the guideline value, are supported by a range of other studies including human data, and these are also considered in carrying out a health risk assessment. In order to derive a guideline value to protect human health, it is necessary to select the most suitable study or studies. Data from well conducted studies, where a clear dose-response relationship has been demonstrated, are preferred (WHO, 2004).

According to the World Health Organisation, 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 as of yet, with further research needed.

According to the WHO guidelines for drinking water quality there are two principal sources of information on health effects resulting in guideline value derivations. The first is human health studies but due to the lack of good quality information, the second more common source of information is animal data. This results in a need to extrapolate and has many shortcomings.

A new approach is needed for endocrine disruptors. It was therefore decided to develop a framework for guideline development for endocrine disrupting chemicals as a preventative

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.

This document attempts to look at the best approach to derive drinking water quality guidelines for endocrine disrupting chemicals for South Africa.

8. SETTING GUIDELINE VALUES FOR ENDOCRINE DISRUPTORS

The next section describes the methods available to set guideline values for potential harmful chemicals. A short description of available methods to determine potential health risks associated with endocrine disruptors is given. It also deals with the current health risk assessment process and how it is traditionally used to develop guideline values for chemicals in water. Finally the uncertainties or drawbacks in terms of endocrine functioning are discussed.

8.1 Precautionary Approach To Handling Endocrine Disrupting Chemicals

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. It also implies if there is a slight suspicion that endocrine disrupting chemicals might have negative health effects in humans from drinking water, that guidelines for these chemicals in water should be derived to prevent potential negative health effects in humans. The precautionary principle seeks to trigger action or reaction in advance (pro-active) before any irreversible damage to human health occurs (Burger, 2003).

The precautionary principle approaches environmental and public health policy decisions from a vantage point in conflict with the traditional position. Traditionally, we base our decisions about environmental policy on acceptable risk levels. The precautionary principal's guiding rule declares that we are obliged to initiate precautionary or preventive measures when a specified activity threatens to harm human health or the environment, even if a direct cause-and-effect linkage cannot be demonstrated unmistakably (Weiss, 2001).

8.2 Health 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 released from specific sites. Risk is a combination of two factors (Schwab and Genthe, 1998):

- The probability that an adverse event will occur
- The consequences of that event

A logical and systematic framework for evaluating human exposure to environmental pollutants was first formalised by the US National Research Council (NRC/NAP, 1983). Within this framework, determining the risks of a given environmental health outcome involves four distinct, but interacting phases, namely:

- **Hazard Identification** characterises the inherent adverse effects (toxicity/ carcinogenicity) of an agent, e.g. causes cancer, birth defects, poisoning, etc. Hazard identification

establishes whether exposure to a chemical or microbiological agent can cause harm and is generally based on primary data from human epidemiological studies and animal toxicology studies. Once a health hazard has been identified, the remainder of the process encompasses the description of the properties of the hazardous agent, and the identification of both acute and chronic health effects.

- **Dose-Response Assessment** characterises the relationship between the dose of a hazardous agent (i.e. the amount of the substance taken into the body through inhalation, ingestion and dermal contact) and incidence of an adverse effect in the exposed population.
- **Exposure Assessment** measures or estimates the intensity, frequency and duration of human contact with a contaminant in the environment. To determine exposure, it is necessary to combine an estimation of environmental concentrations of the hazards with demographic or behavioural descriptions of the exposed population.
- **Risk Characterisation** provides an indication of the incidence of the health effect under the conditions of exposure described in the exposure assessment and the identified dose-response relationship.

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. Additional or substitute methodology applications are also discussed.

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 toxigenic (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 (WHO, 2004; US EPA, 2002 (a)). This safe threshold is also referred to as the reference dose. Figure 1 shows an example of a non-cancer slope factor.

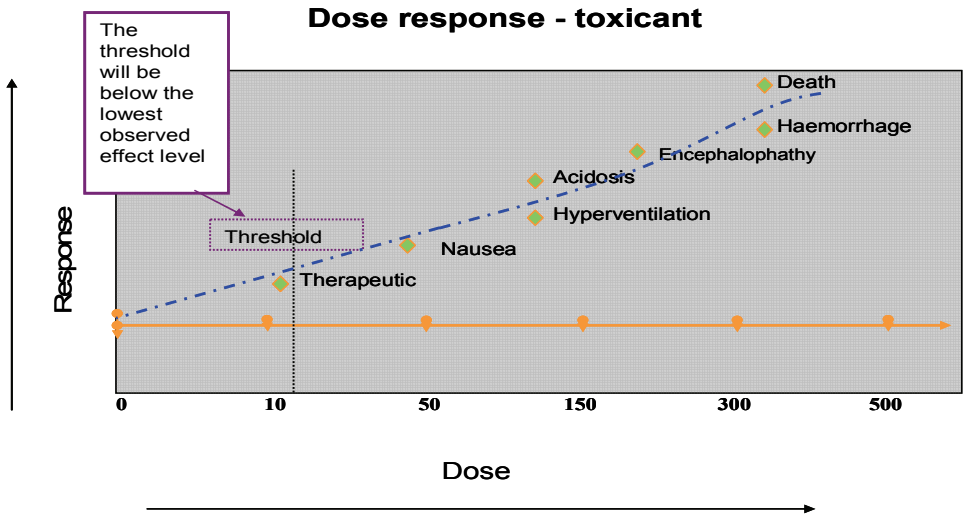

Figure 1: Toxicant dose-response curve

Carcinogenic (cancer-causing) substances, on the other hand, are assumed to have no safe level of exposure (WHO, 2004; US EPA, 2003). The dose-response model used assumes that at low concentrations a straight line can approximate the relationship between exposure and carcinogens and that it passes through the origin. This means that it is assumed that an exposure to even a very small amount of carcinogen will result in a potential risk. Because these chemical compounds are considered unsafe at any concentration, the quantitative human health risk for cancer effects is expressed in terms of a probability, or cancer risk, rather than as a hazard quotient that is either "safe" or "unsafe". An example of a cancer dose-response curve is depicted by Figure 2.

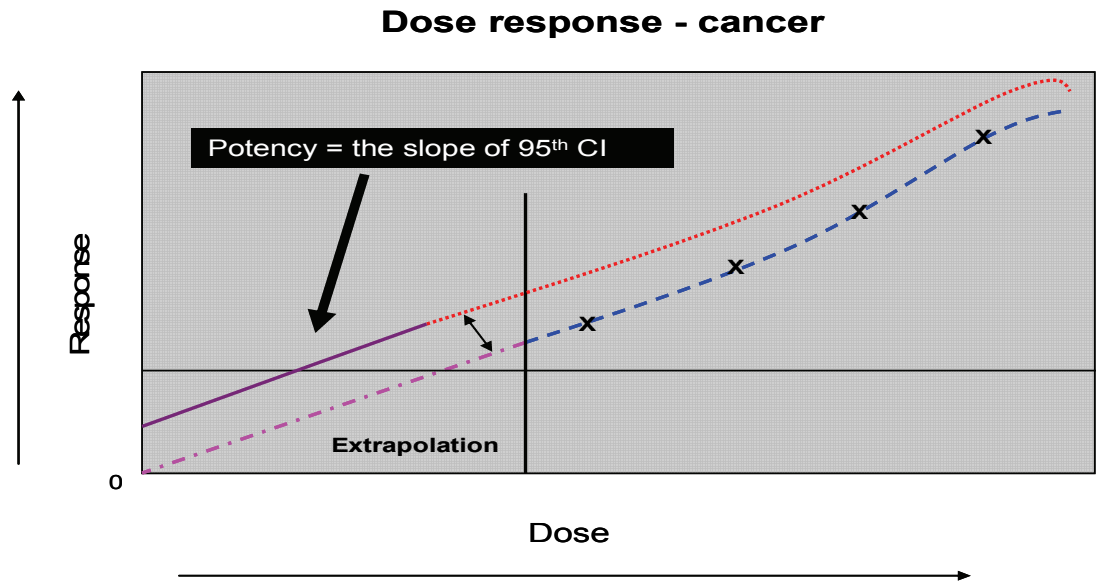

Figure 2: Cancer slope factor

Conventional toxicological experiments have been based on the assumption that "the dose makes the poison", which implies that high dose invariably causes more harm than lower doses. Contrary to the above hypotheses, responses to hormones are different. High doses of hormones and endocrine disrupting chemicals can block rather than stimulate some responses, resulting in what is called a non-monotonic dose-response relationship (Myers et al., 2003; Welshons et al., 2003).

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. Welshon 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.

This non-monotonic dose-response relationship is illustrated in the figure below (Figure 3). In the first graph (the inverted U) the effect initially increases and then decreases with increasing dose. In the second graph, the reverse occurs.

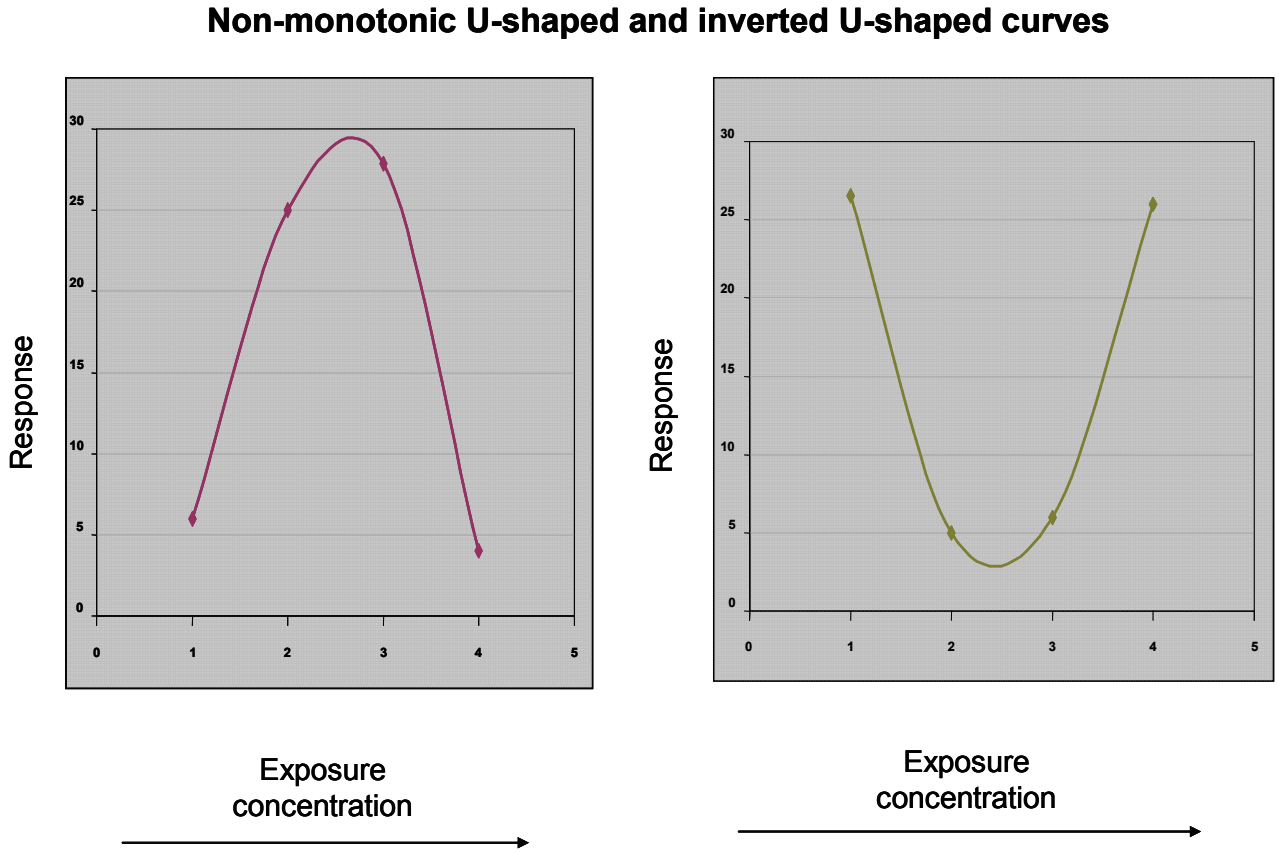

Figure 3: Examples of non-monotonic dose-response curves

Based on various research studies completed internationally, for some endocrine disrupting chemicals there are often no threshold. 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).

9. EFFECTIVENESS OF CURRENT RISK ASSESSMENT METHODOLOGY FOR ENDOCRINE DISRUPTING CHEMICAL GUIDELINE DEVELOPMENT

The inability to deal with multiple chemical exposures to derive meaningful predictions of human health effects is a profound weakness in this and all human health risk assessments to date. The National Occupational Research Agenda (NORA) stated that mixed exposures may produce acute or chronic effects or a combination of acute and chronic effects, with or without latency. Other exposures in combination with certain stressors may produce increased or unexpected deleterious health effects, or they may combine or interact in the environment to create a new exposure risk (Klimek, 2005).

Risk assessment should always be based on aggregate risk; such that all exposure routes to the substance are considered. The current human health risk assessment approach for endocrine disrupting chemicals has already been found to have shortcomings, because endocrine active substances (EASs) may behave in an additive manner for some effects, but antagonistic for others (Gies, 2003). Exposure to mixed stressors can produce health consequences that are additive, synergistic, or antagonistic. Mixtures may have substantially different environmental effects than

the sum of individual substances.

As an alternative to developing guideline values for individual chemicals suspected of endocrine disrupting activity a method looking at the ability to activate endocrine systems may be a possible solution until more information is available. This can be compared to the approach used for whole effluent toxicity as implemented by the DWAF (also known as the DEEEP approach, Slabbert et al., 2003).

Despite various pitfalls and the limited database available for endocrine disrupting chemicals, current risk assessment methods are believed to be largely appropriate for assessing the risks associated with hormonally active substances (endocrine disrupting chemicals). However, they may need to be adapted to address unusual aspects of endocrine-mediated effects.

The German Federal Environmental Agency proposed to perform a risk assessment according to procedures agreed for other substance classes, but to apply an additional safety factor of 3 to 5 in human health and environmental risk assessments until the major problems of the methodology of risk assessment for endocrine-disrupting substances are solved (Gies, 2003).

The following properties of endocrine disrupting chemicals influence the ability of the current methodology to assess the human health risks associated with these substances:

- ***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). The ability of epidemiological studies to identify the cause of an adverse effect decreases as the prevalence of the effect and the number of causal factors increase. 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 we therefore have no control (Myers et al., 2003).

- ***Threshold and linear model assumptions***

  It has been assumed that toxic (non-carcinogenic) chemicals have a threshold level of safe exposure, and that dosage effects are linear. However, for some endocrine disrupting chemicals there is no threshold and even extremely low doses cause changes or have damaging effects. It's been found that endocrine disrupting chemicals follow either a U-shaped or inverted U-shaped dose response curve.

- ***Transgenerational properties***

  As early as 1984, Jacobson and colleagues found that endocrine disrupting chemicals can be transferred across the placenta and into maternal milk, thereby affecting their offspring. Although many uncertainties still exist, increasing evidence suggests that endocrine disrupting chemicals definitely have transgenerational effects in animals (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***

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. Based on this, Den Voogt and Van Hattum (2003) voiced the need for a life-stage specific prediction exposure model.

The pharmacokinetics of chemicals is markedly different during foetal and early postnatal life relative to adulthood. Similarly, pregnant and non-pregnant women also differ in this regard. Therefore, dose ranges and responses in pregnant females and foetuses cannot be assumed similar to those in adults and should be evaluated separately (Welshon et al., 2003).

- ***Synergistic and additive effects between chemicals***

In reality, humans are not exposed to a single chemical, but a mixture of such chemicals and the possibility that such chemicals have additive or reinforcing effects has to be considered. Since standard animal tests to evaluate these effects would be extremely complex with many potential problems, alternative approaches for epidemiological methods in humans and animals need to be developed (Royal Society, UK 2000).

Most toxicological studies examine the effects of only a single chemical at a time, however, 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 with regard to 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 estrogenic 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). Much work remains to be done on the study of the human health impact of exposure to endocrine disruptors, particularly in view of the exposure experienced by people to mixtures of these chemicals (WHO, 2003).

- ***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 effects on for example male reproductive organs, since chemicals with oestrogenic or anti-androgenic activity may have reproductive effects in males. The level at which these chemicals act may not be the same across species, however, the mechanism of action for these endocrine disrupting chemicals will be consistent, or similar, across species.

Potentially important properties of endocrine disrupting chemicals that need to be considered in studying these chemicals, are their ability to accumulate in the body or alter the production or metabolism of endogenous sex steroids (Royal Society, UK, 2000).

▪ *In vitro versus in vivo testing*

For many environmental chemicals, we rely on information derived from experimental animal models and laboratory studies. *In vitro* testing systems are especially useful for screening toxicity and identifying mechanisms of action (Coille et al., 2002; IPCS, 2001; WHO, 2003).

"Cross-talk" may occur between different endocrine systems and may result in systems being affected other than the anticipated one. It is therefore very important to be cautious in extrapolating *in vitro* hormone activity detected to the *in vivo* situation (WHO, 2002; STOTS White Paper, 1998). An endocrine active chemical can show activity *in vitro*, but not in an intact animal or human since *in vitro* tests cannot account for all the factors within an intact organism including absorption, metabolism etc. Care should also be taken when extrapolating results from animals to humans (Haseman et al., 2001; Vom Saal and Sheenan, 1998).

In addition, data is lacking with regards to dose response information for endocrine disrupting chemicals.

Assessment of affinity to receptor preparations **alone** does not allow conclusions to be drawn regarding endocrine efficacy. In vitro assays for oestrogenic activity often measure discrete biological responses at cellular level. They do not take into account the absorption, disposition, metabolism, excretion, bioaccumulation, and repair processes in the intact organism, which play a crucial role in the actual toxic response of a chemical (Reel et al., 1997 cited in Kroes et al., 2000).

Toxicokinetics: The process of the uptake of potentially toxic substances by the body, the biotransformation they undergo, the distribution of the substances and their metabolites in the tissues and the elimination of the substances and their metabolites from the body. Both the amounts and concentrations of the substances and their metabolites are studied. The term has essentially the same meaning as pharmacokinetics, but the latter term should be restricted to the study of pharmaceutical substances (www.fsra.net/glossary.html)

Toxicogenomics: The collection, interpretation, and storage of information about gene and protein activity in order to identify toxic substances in the environment, and to help treat people at the greatest risk of diseases caused by environmental pollutants or toxicants.

The use of genomic biomarkers to evaluate toxic effects has been termed "toxicogenomics." Although originally viewed as the use of genomic data to interpret and understand toxicological findings, the definition of toxicogenomics has gradually evolved to encompass other fields. One commonly used definition of toxicogenomics is as follows:

a scientific field that elucidates how the entire genome is involved in biological responses of organisms exposed to environmental toxicants/ stressors. Toxicogenomics combines information from studies of genomic-scale mRNA profiling (by microarray analysis), cell-wide or tissue-wide protein profiling (proteomics), genetic susceptibility, and computational models to understand the roles of gene-environment interactions in disease. (National Center for Toxicogenomics, 2000).

Toxicogenomics are expected to be a useful method in understanding mode-of-action. It can provide us with helpful data relevant to difficult areas such as dose-response relationships, species-to-species extrapolation, and exposure assessment that cannot be resolved with traditional toxicological techniques (Shirai and Asamoto, 2003).

▪ *Inbred strains and laboratory conditions*

Standard toxicological assays traditionally examine animals in the lab but several findings indicate that testing endocrine disrupting chemicals only in lab conditions will fail to detect

effects that occur under more realistic ecological conditions. Risk assessment as currently practiced, usually only evaluates the effects of an individual chemical on a previously unexposed lab animal, but in the real world an animal may be exposed to a variety of substances via several exposure routes as well as from remobilisation of contaminants already stored in the organisms' adipose tissue. Researchers should be cautious about generalising results from a single strain and they should be aware that there are many ways that harmful effects of endocrine disrupting chemicals can go undetected in standard toxicological tests.

- ***Parent products versus metabolites***

Because endocrine disrupting chemicals are structurally diverse and possess quite different physical and chemical properties, their transport, fate, transformation, bioaccumulation and biomagnification need to be studied in order to understand their differential effects at different trophic levels (Den Voogt and Van Hattum, 2003). For instance, endocrine disrupting chemicals possess differential lipophilicity or hydrophilicity resulting in different retention rates and bio-availability in nature. For example, though chemicals such as DDT and nonylphenols are less potent than endogenous hormones and synthetic hormones , their lipophilicity allow them to exist for a prolonged period of time in ecosystems and be passed on to higher trophic levels. Their long term, chronic effects and biomagnification may have more important implications than those of short half-life chemicals (Huang et al., 2003).

10. MEASUREMENT TECHNIQUES OF ENDOCRINE DISRUPTION IN WATER

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 bioassays.

The former 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 approach (2003) followed by the National Toxicity Monitoring Programme that was initiated by DWAF.

Bioassays are valuable tools to measure total estrogenic and androgenic activity resulting from all the endocrine disrupting chemicals present in a water body, including unknowns. Both biological (*in vivo and in vitro*) and biochemical (*in vitro*) methods are used to determine endocrine disrupting chemicals activity and effects. Occurrence of individual chemicals is determined by chemical analysis. 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).

The screening of endocrine disrupting chemical activity is mostly made by *in vitro* methods. *In vitro* methods determine the interaction of a chemical with the endocrine system at cellular level using, for example, cell cultures or enzymes based on the binding of the endocrine disrupting chemicals to a specific receptor.

In vivo experiments, on the other hand, measure endocrine disrupting chemical effects in the whole

animal by measuring a variety of endpoints such as the increase in uterus weight. The major advantage of this type of methodology is that it takes into account absorption, metabolism and excretion. However, *in vivo* test methods are expensive and time consuming and often require the sacrifice of test animals.

▪ In vitro screening methods

Various *in vitro* methodologies are used world wide. These include the yeast estrogen screen (YES) assay, the two-hybrid recombinant yeast cell bioassay (TRCBA), the estrogen receptor (ER) binding assay, the enzyme linked immuno-assay (ELISA), the E-screen cell proliferation assay, the ER-CALUX assay, the DR-CALUX assay, the Carp-HEP assay, and the T47D-KBluc cell line, to mention a few. They can be divided into three categories of assays, depending on which endpoint of biological response they measure (Kinnberg, 2003):

a) receptor binding assays

b) reporter-gene assays

c) cell proliferation assays

Only a few of the abovementioned *in vitro* methods are currently practiced in South Africa:

10.1 The Recombinant Yeast (YES) screen assay (Routledge and Sumpter, 1996)

This rapid, cost-effective and widely used assay is based on modified yeast cells which possess the human oestrogen receptor. The activated receptor binds to the oestrogen response element located on a reporter plasmid, in tandem with a sequence coding for β-galactosidase. In the presence of estrogenic agents the cells begin to express β-galactosidase. The enzyme is excreted into the culture medium where it reacts with its substrate CPRT to liberate chlorophenol red. The resulting colour change from yellow to red is readily measured spectrophotometrically, compared to a standard curve and the estrogenic potency of the sample expressed as “Estrogen Equivalencies” (EEq).

10.2 MVLN assay (Demirpence et al., 1993)

This reporter gene assay measures growth of cells in response to agents that interact with the estrogen receptor, and is also sensitive to agents that do not interact with the protein (Pons et al., 1990). The assay utilises MCF-7 breast cancer cells (Soule et al., 1973) that has been stably transfected with the Vit-Luc reporter gene (Pons et al., 1990). The MVLN cell line expresses the endogenous estrogen receptor of MCF-7 and at the same time contains an exogenous estrogen responsive reporter (luciferase). Therefore, the estrogen specific transcription activity of a test chemical is directly related to the activity of luciferase measured in the lysate of treated MVLN cells. (Gagne et al., 1994; Pons et al., 1990; and EDSTAC, 1998). The use of this screen has not yet been validated.

10.3 E-screen (MCF-7 cells) assay (Soto et al., 1995)

The E-screen is a cell proliferation assay. These assays are generally based on, either breast cancer- or genetically engineered- cell lines that require estrogen for growth. The assay compares the number of cells present after a specific duration of exposure (e.g. five days) to an estradiol standard curve. The MCF-7 cell line is normally used, although the T47-D cell line is equally sensitive. A constant source of estrogen free water to use for controls remains a problem. It is also more expensive and time-consuming than other in vitro methods and therefore limits its use for large-scale screening programs. This method has been successfully implemented by the

Department of Urology, at University of Pretoria.

10.4 Liver slice HEP-Vtg (Shilling and Williams, 2000)

Instead of using isolated cells, this reporter gene assay can also be performed using liver slices (trout or Xenopus laevis), which is more representative of the organ in vivo. This method is similar to the fish HEP-Vtg, except that it uses liver slices instead of hepatocytes. Vitellogenin is quantified by ELISA at the end of the exposure period.

▪ In vivo screening methods

The *in vivo* assays presently suitable for monitoring in South Africa are the zebra fish assay and *Xenopus lavis* assay for determining estrogenic effects. These methods are however, very expensive and time consuming.

10.5 Catfish Vitellogenin assay

Vitellogenin (Vtg) is the most widely used biomarker of exposure to estrogenic chemicals in fish. The most common way of measuring Vtg induction is the ELISA (enzyme-linked immunosorbent assay). The methodology using catfish Vtg coupled with ELISA detection technique has been developed and successfully implemented at the University of Pretoria, Department of Urology.

Except for the MVLN method that has not been validated yet, all the other screening methods have been successfully developed and implemented and can be performed at various organisations in South Africa (e.g., Department of Urology at University of Pretoria, Department of Zoology at University of Stellenbosch, and the CSIR).

11. WHAT FRAMEWORK FOR GUIDELINES FOR ENDOCRINE DISRUPTORS HAVE BEEN PROPOSED IN OTHER COUNTRIES?

The WHO (IPCS, 2002) has not proposed a framework for guideline development for endocrine disrupting chemicals, but only a framework for assessing the causal relationships of endocrine disruptors. The majority of the scientific knowledge available regarding potential effects of exposure to endocrine disruptors has been collected from North America, Europe and Japan. The potential risks posed by endocrine disruptors in developing countries have not been adequately addressed. However, it is often in developing countries that people are exposed indiscriminately to endocrine disruptors.

Internationally, countries are in agreement that precautionary action should be taken. However, some differ in their approach to precautionary measures. Quantitative human health risk assessment frameworks to estimate the impacts from endocrine disruptors have been proposed by various organisations and from countries globally. These proposed frameworks are more or less the same but vary somewhat in approach between risk-based and hazard-based. A hazard-based approach, also referred to as the precautionary approach, provides the basis for taking preventative measures with respect to substances due to their potential to cause harm to the environment or human health. This entails **using preventative measures prior to assessing the impact or potential risk of the substance** on the environment and human health. On the contrary, risk-based approaches depend on the availability of absolute proof of harm to the environment or human health before action can be taken (Klimek, 2005).

The section below gives a brief overview of the action taken globally by different organisations:

The EU has adopted a community strategy in 1999 to address the potential for environmental and health impacts of endocrine disruption. The community strategy framework is divided in short, medium and long terms goals. Their short- to medium- term goals are to identify substances, start monitoring, research, international co-ordination and communication to the public. The long term actions include reviewing and adaptation of their existing legislation and policies regarding testing, use and production of chemicals in Europe.

As part of the actions taken in Europe a group has been set up for the Registration, Evaluation, Authorisation and Restriction of Chemicals called REACH. EDEN, FIRE, COMPRENDO, EURISKED all in a cluster of research into endocrine disruption in Europe – CREDO cluster.

In the US, the National Science and Technology Council's (NSTC) Committee on Environment and Natural Resources (CENR) already in 1996 developed a framework for planning, chaired by the Environmental Protection Agency (EPA) regarding the health and ecological effects of endocrine disrupting chemicals.

Within the Netherlands, the proposed framework by RIVM is a quantitative risk assessment based on a precautionary principle (Klimek, 2005). RIVM recommend the "effects approach" as applied by the WHO (2004) and USEPA (2002) for risk assessment of chemicals, whereby they differentiate between genotoxic (non-threshold) and non-genotoxic (threshold) effects.

From literature it is evident that it is impossible to derive an over-all guidance value for maximum permissible oestrogenic activity. Instead a trigger value is calculated. This trigger value is based on various findings and assumptions made during human health risk assessments and is similar to a precautionary approach. Instead of determining a TEF (Toxicant Equivalency Factor) for oestrogen activity in water, they derive an EEQ ('Estrogen Equivalent'). This value is derived for oestrogenic activity of estradiol activity in water by means of an *in vitro* bio-assay, namely the ER CALUX method in water (RIVM, 2004).

In the US, the EPA's Guidelines for reproductive toxicity risk assessment (1995), the "benchmark dose" method is recommended to the agency as an alternative to determining a "no-observed adverse effect level" (NOAEL) or "lowest-observed adverse effect level" (LOAEL). Disagreement still exists whether the EPA applies a precautionary (hazard-based) or risk-based approach.

In 2003 Environment Canada states that large uncertainties are associated with any scientific assessment of endocrine disrupting chemicals. They follow the weight-of-evidence approach which relies on the most credible information. They believe that a tiered-testing approach that considers both hazard and exposure is advantageous and will be followed once validated and incorporated internationally (Servos et al., 2002a and b). Australia has proposed the use of the precautionary principle based on a no-observed-effect-level or NOEL.

12. RECOMMENDED SOUTH AFRICAN FRAMEWORK

The suggested or proposed framework for endocrine disrupting chemical guidelines for South Africa is based on a first level screening test for reproductive endocrine disrupting capability rather than for individual chemical concentrations. Should endocrine disruption be detected, then a more detailed assessment is recommended.

12.1 Screening

A screening test using a battery of *in vitro* and *in vivo* tests quantitatively expressing the results of endocrine 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 estrogen equivalency

factors. A value above which a more detailed assessment is recommended would be the "trigger value". This trigger value might be 0.5 ug/L estrogen equivalents – based on a formula for water quality guideline calculations described in more detail in the section below.

▪ Calculation of estrogen equivalent trigger value

The equation to calculate the trigger value is as follows:

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

where the Guideline value concentration in water can be expressed as the trigger value)
ADI is acceptable daily intake 200 pg/kg bodyweight -calculated making use of most current data available (RIVM, 2004).
Bw = body weight – in South Africa 65 kg is average
P = fraction of ADI allocated to drinking water taken as 10% by WHO
IR = intake rate – 2 L

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 uncertainty factors to the toxicity data to take into account differences in sensitivity to toxic effects within and between species, and differences in toxic effects between chronic and subchronic exposure. This may be in the order of 1 up to 10 000 (WHO, 2004; IPCS, 1994). In the case of 17ß-estradiol the uncertainty factor used was 100 (RIVM, 2004).

For the derivation of 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.

In most cases this 10% allocation is sufficient to account for additional exposure through the various routes (e.g. inhalation and dermal absorption) (IPCS, 1994). A proportion of 10% is allocated to water for consumption and food preparation purposes. The other 90% is accredited to food, air, and soil. This low proportion allocated to water as a source of pollution adds extra safety to the guideline (WHO, 2004).

The calculated trigger value is therefore as follows:

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

$$\cong 0.7ug / L$$

12.2 Detailed assessment

If the 'trigger value' is exceeded in the screening test of the water sample it is recommended that a more detailed investigation be carried out. This would entail a more expensive second round of

testing at the individual chemical level and give more detail as to the levels of exposure. No guideline values can be given at this stage; however this detailed assessment would allow mitigating measures to be taken to reduce the levels of the chemicals responsible for endocrine disruption.

13 SUMMARY

It is acknowledged that the proposed approach described in this document has severe shortcomings. We are (as is the rest of the world) trying to propose a method to protect human health. A start must be made with regards to controlling possible endocrine disrupting chemicals in our water. We will need to reassess the approach as new methods and more data becomes available. The problem of developing guidelines for endocrine disruptors in water is not unique to South Africa. As part of the Global Water Research Coalition (GWRC), an organisation comprising 12 international research organisations formed to promote international cooperation and collaboration in water-related research. **Endocrine Disrupting Compounds** were selected as the first item of research cooperation. A research planning workshop was organised to exchange available information within the GWRC membership and to identify the knowledge gaps and research needs. Research projects about the occurrence and fate of endocrine disrupting chemicals during wastewater treatment and sewage and soil are in progress.

In summary, endocrine disrupting chemicals occur in South African waters and we need a process to respond to this without waiting for perfect data. At this stage we are not certain at which levels endocrine disruptors adversely affect health. In addition it is not practical to test for each individual chemical that may cause endocrine disruption. We therefore need to make use of methods that test for endocrine disrupting activity.

It is recommended that a battery of tests eventually be included, and that these tests include a number of taxa. We will also need to include endocrine effects other than reproduction such as thyroid and immune effects. One of the more difficult problems we will need to address is the trans-generational effects of endocrine disruptors.

Research on this problem will continue and new values will become available as our understanding and information improves. It is recommended that this proposed framework be tested for its feasibility within the domestic water quality arena.

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