Monitoring and Assessment of Endocrine Disrupting Chemicals

Report to the
Water Research Commission

by

J A Meyer[1], B Genthe[2] and J Chamier[2]

[1]University of Pretoria

[2]CSIR Stellenbosch

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Obtainable from:
Water Research Commission
Private Bag X03
Gezina, 0031

orders@wrc.co.za or download from Website: www.wrc.org.za> Knowledge Hub > Special Publications

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EXECUTIVE SUMMARY

INTRODUCTION

The Water Research Commission (WRC) has funded Endocrine Disruptor Chemical (EDC) and Toxicant research from 2001. The funding programme has produced numerous research project publications that address different Key Strategic Areas within the WRC. As these are technical reports the WRC decided to develop a Manual on Endocrine Disrupting Chemical Management in Water Resources in a more user friendly format that would reach a wider target audience. The Manual takes the form of several Volumes, with the aims of synthesizing available research, stimulating and assisting on-going research regarding multidisciplinary fields relevant to EDCs. The Volumes are intended to serve as active documents that may be updated as new information emerges.

The current series of Volumes in the Manual are:

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Volume 5: EDC Management in Catchments (WRC Report No. TT 562/13)

This Volume (4) will focus on Monitoring and Assessment but as with all the Volumes is intended to serve as a guide and to provide reference material regarding EDCs in water. The Volume should thus assist a wide target audience, including water supply agencies, water resource managers, workers in health and water-related fields, educators and communities in South Africa.

BACKGROUND AND JUSTIFICATION

The presence of EDCs in source waters, drinking waters and wastewater is of international concern. The potential adverse effects following exposure may impact on human health and animal health by disrupting the endocrine system and affecting a variety of endpoints, ranging from developmental to behavioural aspects. The oestrogenic effects of EDCs have received the most attention but additional endocrine pathways are recognized as being disrupted by EDCs and the current international research focus includes many other endocrine endpoints.

As detailed in the National Water Act (No. 36 of 1998) the water resource classification system balances competing priorities for access to water quality and quantity. As causal links between exposure to EDCs in water and endocrine endpoints become increasingly established it follows that formulating resource quality objectives to define the desired management class also requires guidance in terms of EDCs present in water.

Although various aspects of this are dealt with in the different Volumes of the Manual the inputs to the processes defined therein rely on the ability to detect, screen, perform appropriate analytical techniques and assess the potential effects following exposure to EDCs.

The Manual is intended to be of application for a range of established EDC effects, namely oestrogenic, androgenic, antiandrogenic, thyroidogenic and steroidogenic. This list is not intended to be all-inclusive, nor is it intended to include suspected endpoints in great detail.

Since new mechanisms and systems affected by an ever increasing list of EDCs are continually being reported and investigated it is not within the scope of any of the Volumes to be all-encompassing regarding EDCs. Additional chapters or sections may however be added to the Volumes should the need arise. The Volumes represent a synthesis of the most salient aspects from numerous WRC reports and research project deliverables. There are also several international organisations that routinely publish reviews on EDCs and related research topics, for example the Global Water Research Coalition (GWRC). For further detail please go to www.wrc.org.za > Knowledge Hub > Special Publications.

The Volumes also address the current capacity and capabilities in the context of EDCs and water quality in the South African context. The Manual is thus also highlights critical research needs to stimulate and prioritise further EDC research.

PROJECT SUMMARY

The project initially compiled an introduction to EDCs that was to serve as the first chapter of this Volume. As the topic of EDCs continues to engage with more disciplines it emerged that the introduction would be more suited to a Volume on its own. Consequently, only a generic introduction is thus presented in this Volume.

A significant aspect of the project that was investigated related to the current data sources and data quality for EDC detection and monitoring in South Africa. This allowed for a perspective of needs, capacities and capabilities to be determined for the monitoring and assessment of EDCs. These outcomes were then tested in practical site-specific case studies in which monitoring and assessment of EDCs was conducted. The objective was to not only provide an example of how to proceed with the monitoring and assessment of EDCs but to also evaluate the methods presented in the various Volumes of the Manual. The case studies also included, for the first time in the WRC EDC programme, the investigation and presentation of thyroid dysfunction due to potential EDC exposure via the drinking water in a sentinel livestock species. A robust, cost-effective method for investigating several EDC endpoints in a sentinel livestock species was tested and based on the results, proposed as a viable addition to EDC monitoring and assessment. Analytical procedures required to routinely assess inorganic EDCs were also developed and tested for the case studies.

Whilst conducting the case studies the project engaged with several Government Departments, notably the Department of Mineral Resources, the Department of Environmental Affairs, the Department of Agriculture, and the Department of Water Affairs. It was established that in order for the monitoring and assessment of EDCs to have any significant impact legal compliance with the relevant sections of several Acts and Government Notices was essential. Although this related primarily to the manner in which current monitoring requirements were being formulated within the directions and intentions of existing legislation, stakeholder consultation and comments submitted for various reviews and Government Notices, resulted in an invitation to submit proposals for best practice guidelines. Since this was not accommodated in the scope of this Volume, nor the Manual, it remains a recommendation to develop a single guideline document for the Directorate of Water Resource Protection and Waste at the Department of Water Affairs.

The fundamental objective of the project was thus to highlight the approach required for the regulator/ health authorities to make informed decisions based on acceptable scientific data within the context of the challenges posed by the monitoring and assessment of EDCs. As with other the Volumes the approach adopted remains based on the precautionary principle as many of the EDC potential hazards posed are new and not yet fully described.

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WATER RESEARCH COMMISSION

Dr K Murray	Water Research Commission (Chairperson)
Dr JB Molwantwa	Water Research Commission (Chairperson)

RESEARCH TEAM

Project leader

Dr JA Meyer	Department of Animal and Wildlife Sciences University of Pretoria

Senior Partners

Ms B Genthe	CSIR Stellenbosch
Dr J Chamier	CSIR Stellenbosch

Reference Group Members

Prof C de Jager	University of Pretoria
Dr NH Aneck-Hahn	University of Pretoria
Dr IEJ Barnhoorn	University of Pretoria
Dr R Pieters	North West University
Ms MC van Zijl	University of Pretoria
Prof NH Casey	University of Pretoria
Dr E Ncube	Rand Water
Prof H Bouwman	North West University
Dr R Heath	Golder and Associates
Ms L Boyd	Golder and Associates
Dr S Jooste	Resource Quality Services (Department of Water Affairs)

Collaborators

Mr D Hudson-Lamb	Pelinadaba Analytical Laboratories (NECSA)
Dr M Holoi	Cape Town Scientific Services
Ms JE van Rooyen	Institute for Soil, Climate and Water (ARC)
Mr CM Fouche	SABS: Food and Water Chemistry
Mr J Parsons	Rand Water
Prof N Duncan	Department of Paraclinical Sciences (University of Pretoria)

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LIST OF ABBREVIATIONS AND ACRONYMS

ADD	Acceptable Daily Dose
ADI	Acceptable Daily Intake
AE	Adverse Effect
EDC	Endocrine Disrupting Chemical
ER	Estrogen Receptor
GWRC	Global Water Research Coalition
IPCS	International Programme for Chemical Safety
OECD	Organisation for Economic Co-operation and Development
RfD	Reference Dose
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation
WRC	Water Research Commission

A full list of abbreviations and acronyms are presented in Volume 1 of the EDC Manual.

USEFUL CONTACTS

The Pelindaba Analytical Laboratory contacts are: **Ms Lenie Heystek** Manager PAL (012) 305 4325 082 308 0521 Lenie.heystek@necsa.co.za **Mr D Hudson-Lamb** Senior Scientist – PAL (012) 305 4126 082 809 5637 Dave.hudsonlamb@necsa.co.za **Ms M Gerber** Section Head – Trace Lab (012) 305 4237082 304 0586 Magda.Gerber@necsa.co.za Website: www.necsa.co.za	The Institute for Soil Climate and Water contact details are: **Mr T Matema** Head: Water Laboratory (012) 310 2500 matemat@arc.agric.co.za **Ms JE van Rooyen** Senior Technician: Water Laboratory (012) 310 2500 annatjie@arc.agric.co.za **Ms AM du Preez / Ms N Sotaka** Senior Technician: Soil, Plant & Tissues (012) 310 2500 adupreez@arc.agric.co.za
The SABS Contact details are: **Mr J Morobi** Manager: SABS: Food & Water Chemistry (012) 428 6844 jabu.morobi@sabs.co.za **Ms S Mabe** Senior Test Officer (012) 428 6745 Sannie.mabe@sabs.co.za	The Bioassay Contact details are: Oestrogenic: **Dr N Aneck-Hahn** Director: Environmental Chemical Pollution and Health Research Unit Department of Urology Steve Biko Academic Hospital University of Pretoria Natalie.Anneck-Hahn@up.ac.za naneckha@medic.up.ac.za **Mrs C van Zijl** Department of Urology Steve Biko Academic Hospital University of Pretoria Catherina.vanzijl@medic.up.ac.za Androgenic: **Dr R Pieters** Unit for Environmental Sciences and Management North West University Potchefstroom Rialet.pieters@nwu.ac.za
The Human Risk Assessment Contact details are: **Ms B Genthe/Dr J Chamier** Environmentek CSIR Stellenbosch b.genthe@csir.co.za	

BACKGROUND

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This Volume (4) will focus on Monitoring and Assessment but as with all the Volumes is intended to serve as a guide and to provide reference material regarding EDCs in water. The Volume should thus assist a wide target audience, including water supply agencies, water resource managers, workers in health and water-related fields, educators and communities in South Africa.

The various aspects relating to the management of EDCs in catchments that need to be addressed are dealt with in the different Volumes of the Manual. As a general overview of the process it may be observed that the inputs defined therein rely on the ability to detect, screen, perform appropriate analytical techniques and assessments of the potential effects following exposure to EDCs. Outputs from these processes are then used in conjunction with other management linked functions to formulate risk management strategies and consistent, meaningful communication regarding the hazards and risks posed by EDCs.

As there is a growing global concern about the scope of hazards and risks posed by EDCs, with the possibility of serious adverse effects reported in humans and animals, persistence of some EDCs and suspected EDCs in the environment, research on EDCs is generally given a high priority. Due to the multi-disciplinary approach that is required when conducting studies on EDCs different investigations may prioritise these concerns differently. In some instances consensus has yet to be reached regarding the physiological significance in terms of adverse effects of various forms of endocrine disruption. It remains necessary the interim to develop strategies to monitor and assess EDCs in order to manage EDCs in catchments, from release, removal to mitigation of risks.

As is noted in Volume 5 of the Manual, certain questions need to be answered in order to make informed and rational decisions with regards to EDCs. These include:

- What are EDCs and what do they do;
- How are they detected and at what values are they of concern;
- How much certainty, or uncertainty, is attached to the detection, screening and estimated risk of EDCs;
- What actions can be taken and how should they be evaluated;
- How should these actions be communicated?

This Volume will address some of these questions and Volume 1 may be consulted for links between various shared components in the different Volumes.

1. INTRODUCTION

This Volume provides an introduction to concepts and applications of the monitoring and assessment of endocrine disrupting chemicals (EDCs) as relevant to the management thereof in water resources. It is emphasized that the level of detail provided is not intended to be overly technical, nor is it to cater for specialist information needs. The content provided was determined following consultation with a wide range of relevant target audience representatives with references to more detailed reviews and scientific literature provided in each of the Volumes.

This Volume first provides an introduction to EDCs in order to allow the user to understand the topic and context for which the Manual has been developed. The topics of Monitoring and Assessment are then presented in order to allow the application of the outcomes from the other Volumes in the series to be placed in context.

A summary of two case studies is also presented in which the various relevant processes are broadly outlined, more to provide an example of the sequencing used for the specific objectives that relate to each site than to be prescriptive.

It is not within the scope of any of the Volumes to be all-encompassing regarding EDCs. The Volumes represent a synthesis of the most salient aspects from numerous Water Research Commission (WRC) reports and research project deliverables, with additional contributions to be added as the need arises. There are also several international organisations that routinely publish in-depth technical reviews on EDCs and related research topics, for example the Global Water Research Coalition (GWRC), and the reader is referred to the reference list for additional information. For further detail please go to www.wrc.org.za > Knowledge Hub > Special Publications.

The chapters of this Volume also contribute to the overall objective of the Manual, namely to guide and stimulate research and hence increase the amount of valid data available from which credible risk assessments may be performed, and in so-doing, be able to implement management actions based on informed judgments. This is done with due regard for the current capacity and capabilities in the context of EDCs and water quality in the South African context.

2. ENDOCRINE DISRUPTING CHEMICALS

2.1 Introduction to the Field of Research

Over the last three decades scientific statements have been expressing increasing concerns about potential adverse effects (AE) that may result from exposure to a group of chemicals with specific reference to their potential to change the functioning of the endocrine system.

The awareness that chemicals in the environment can have AEs on wildlife, and that human health is inextricably linked to environmental health, was highlighted by the publication of Rachel Carson's *Silent Spring* [6].

These concerns have arisen primarily due to the observation and reporting of AEs in some wildlife species, fish and ecosystems. In some cases these chemicals are been reported to increase endocrine-related human diseases. Laboratory experiments have also demonstrated endocrine disruption to occur following exposure to some environmental chemicals.

Toxicological and carcinogenic effects are recognized following exposure to a comprehensive list of water quality constituents [2, 3, 4, 5, 6]. This series deals specifically with the potential of chemicals present in water to interfere with the endocrine system, a distinction covered in more detail in section 2.2.

Generally these chemicals have been given the term Endocrine Disrupting Chemicals, but some variations on this theme exist.

The publication of the hypothesis that xenobiotic chemicals (chemicals not produced in the body, taken from the Greek "xenos" meaning stranger) used and released into the environment could disrupt the endocrine system of mammals and wildlife used the term "endocrine-disrupting contaminants" [7].

The United States Environmental Protection Agency (US EPA) uses the term "Endocrine Disrupting Compounds" whilst the World Health Organisation prefers the term "Endocrine Disrupting Chemicals".

Both the Endocrine Society of the USA [8] and the International Programme on Chemical Safety [9] uses the term "Endocrine-Disrupting Chemicals". The Global Water Research Coalition uses both compounds and chemicals to imply endocrine disruptors [10].The generic acronym EDC is used in this manual.

The WRC has been investigating these concerns as they relate to water, water users and water uses for over a decade.

Although the topic is contentious with conflicting scientific opinions having led to much public debate this Manual concerns itself with scientifically accepted methods for sampling and conducting bioassays and analytical determination in order to appropriately monitor, assess and manage EDCs in Water Resources.

As a member of the GWRC the WRC of South Africa has been involved in a collaborative international water research alliance since 2002. The GWRC focus is more on the urban water cycle than at the catchment level, but renewable resources also form part of the focus areas. In a recent review on endocrine assays [10] it was observed that many of the bioassays used to test hormonal activity had not been fully tested for water samples and that such testing would be of great benefit to allow for meaningful application thereof to water testing. It was also noted that most of the assays testing endocrine activity had been applied to patchily to wastewater and that further information is required for other waters, such as drinking water, groundwater and surface water. It is clear that not all endpoints affected by EDCs are equally understood or researched, but EDCs are regarded as posing a serious threat to human and animal health. Despite it being stated that "It is still unclear whether the concentrations detected are sufficient to case significant endocrine disruption", it concludes that "Nevertheless, endocrine disruption is a potential serious threat to human life even at low concentrations, and it is important to understand the contribution from water and its relevance." On-going efforts are required to understand the significance of the role played by water in endocrine disruption.

The reader is referred to the list of WRC reports and other publications in the References section for further detail on the EDC topic and issues presented in this Manual.

2.2 What is an EDC?

A chemical can be defined as a substance with a distinct molecular composition that is used in or results from a reaction involving changes to atoms or molecules.

A compound is defined in the field of chemistry as a pure, macroscopically homogeneous substance consisting of atoms or ions of two or more different elements in definite proportions that cannot be separated by physical means with properties usually unlike those of its constituent elements.

The term "disrupt" may be interpreted in the context of physiology to imply that a biological process has been interrupted to the extent that it has either been stopped, or been prevented from normal continuance.

The term "endocrine" relates to a specific system within the body where internal secretions occur that are distributed in the body via the bloodstream. It may also be taken to refer to hormones. An endocrinologist studies mainly the physiology and pathology of internal secretions and endocrine glands.

In a World Health Organisation (WHO) global assessment on endocrine disruption the International Programme for Chemical Safety (IPCS) defined an endocrine disruptor as [9]:

- 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.

Since many reported instances of endocrine disruption have not yielded unequivocal evidence of adverse effects this definition may be modified to read:

- an exogenous substance or mixture that alters function(s) of the endocrine system and consequently *may cause, or potentially result in*, adverse health effects in an intact organism, or its progeny, or (sub)populations.

The IPCS went on to define a potential endocrine disruptor as [9]:

- an exogenous substance or mixture that possesses properties that might be *expected to lead to endocrine disruption* in an intact organism, or its progeny or (sub)populations.

The initial definition of an endocrine-disrupting compound by the US EPA was [8]:

- an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction and developmental process.

The Endocrine Society acknowledged in 2009 that the understanding of the mechanisms by which endocrine disruptors exert their effects has grown [8], and now recognizes much broader mechanisms. Thus, in addition to nuclear hormone receptors (e.g. oestrogen and androgen receptors, progesterone receptors, thyroid receptors) effects "via nuclear receptors, non-nuclear steroid hormone receptors (e.g. membrane ERs), nonsteroidal receptors (e.g. neurotransmitter receptors), orphan receptors (e.g. aryl hydrocarbon receptor), enzymatic pathways involved in steroid biosynthesis and/or metabolism, and numerous other mechanisms that converge upon endocrine and reproductive systems" are also recognized, with the definition accepted from a physiological perspective as:

- an endocrine-disrupting substance is a compound, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment".

Recently, a review of bioassays by the GWRC [10] notes efforts are expanding to develop and validate methods to study and measure the less well-studied endpoints, such as thyroid, glucocorticoid and retinoid systems. These less well-studied endpoints were also identified by the

OECD as a priority for endocrine disruption research. It should be noted that observations linking EDC exposure to causal links are not necessarily relevant to exposure from EDCs in water.

New information on the nature of these types of adverse effects and the proposed mechanisms involved are thus continually being researched and published for different fields of application, not necessarily related to water. Accordingly, the definition of an EDC may be redefined with the emergence of new information and to better fit a working definition of the intended field of application.

2.3 Who Researches EDCs?

It has been observed that EDCs present the scientific community with less of a new field, but rather more of an integration of numerous multidisciplinary fields [11]. These are too numerous to list but include biology, chemistry, epidemiology, and atmospheric and earth sciences.

As scientists strive to explain the complexities of our impact on the environment increasing collaboration between different fields has led to new understanding and inevitably new questions, theories and hypotheses. It is therefore not surprising that a single study cannot provide all the necessary information on EDCs, and that there are continual assessments of various aspects of EDCs published by collaborative groups.

It is also noteworthy that research on EDCs is not confined to the endocrine events in receptor species, but that a significant amount of published EDC research deals with detection and screening methodology. Research also extends to the regulatory aspects and may thus involve regulators and water resource and environmental managers.

2.4 Basic Principles Applicable to EDCs

Although differences exist between chemicals that may disrupt the endocrine system and those chemicals that act as poisons (toxicants), or that may be administered to achieve a beneficial effect (therapeutic drugs), many similarities also exist and by way of introduction it may be useful to consider some basic principles of the fields of toxicology and pharmacology first.

Toxicological studies investigate the properties of toxicants as well as their biological effects. Pharmacological studies investigate substances that interact with living organisms through chemical processes, especially by binding to regulatory molecules and activating or inhibiting normal body processes. Both fields of study are applied to prevent, diagnose and treat diseases and disorders.

Although the field of pharmacology studies the effects of chemicals (drugs) used at therapeutic doses on organisms whilst toxicology focuses on chemicals that are harmful, both fields overlap significantly. Despite the fields of pharmacology and toxicology seemingly dealing with two opposite effects, namely one beneficial and the other detrimental, both fields require similar information types and use similar concepts.

Studies on endocrine disruption also share many similarities with regard to the fields of pharmacology and toxicology. Three fundamental commonalities exist in these three fields of study, namely:

- Sources
- Pathways
- Receptors

The Monitoring and Assessment section that follows demonstrates how these three commonalities are used to assist with the Management of EDCs.

Prior to discussing endocrine disruption and endocrine disrupting chemicals some key concepts need to be understood. In order to better understand these concepts is it a requisite that the basic functions of organs in mammalian systems are presented first.

2.5 Fundamentals of Physiology

2.5.1 Basic Structure and Systems

Understanding how EDCs can affect humans or other organisms requires an introduction to physiology. Whilst this section does not provide a comprehensive overview of the different types of physiological processes relevant to all organisms, mammalian physiology serves as an appropriate starting point. It is easier to follow physiological processes when the structure to which they apply is known, thus a brief glance of the structure of the mammalian body is presented in Table 1.

The mammalian body carries out complicated and diverse functions such as breathing, eating, reproduction, excretion and adaptation to the external environment.

Specific systems are developed to accomplish these functions. These systems are also connected. As an example the skin comprises two main layers, namely an outer epidermis and a dermis. Some cells originating in the epidermis differentiate to form different glands, such as sebaceous glands and sweat glands, which grow into the dermis, The dermis may contain tissue types from different systems, such as nerve endings from nervous system and capillaries from the blood vascular system.

Since the skin consists of differentiated tissues each performing specific functions it may also be thought of as an organ. The presence of different tissue types and systems within this organ, namely the skin, demonstrate how these systems can be linked and thus present as a complex integrated system.

Hence organs such as the lungs and kidneys with different functions exist in the same linked physiological manner. Some key aspects of physiological systems with reference to endocrine disruption are presented in Table 2.

Table 1. Basic structure of the mammalian body.

Component	Description
Atoms	All matter in different forms (solid, liquid or gas) consists of structural units called atoms.
Elements	The grouping of atoms forms elements, with living matter consisting of four basic elements: carbon, hydrogen, nitrogen and oxygen.
Compounds	Combinations of different elements gives rise to compounds with structural units called molecules. For example a water molecule consists of two hydrogen atoms and one oxygen atom. Compounds may be organic (contain carbon atoms) or inorganic (do not contain carbon atoms). The mammalian body consists of the following basic organic compounds: water, proteins, carbohydrates and fats. To these additional organic compounds are added such as nucleic acids and steroids.
Cells	The combination of compounds forms cells. These are living organisms consisting of complex mechanisms that can perform different functions, such as nutrition, digestion, energy production and reproduction. The mammalian body consists of more than 100 trillion cells.
Tissues	Cells can combine to form specific anatomical elements recognized as tissues. When a group of cells work together in a homogenous fashion they are defined as a ***tissue.*** The main tissue types are: - Squamous Epithelial (skin & mucous); - Secreting Epithelial (exocrine and endocrine glands); - Connective Tissue - Muscular Tissue - Blood Tissue - Nervous Tissue - Lymph Tissue
Organs	Different tissue types combine to form organs that have a specific function or functions in the body, for example the liver and kidneys. An ***organ*** links cells by supporting structures in a complex system whereby different types of cells perform specific functions – referred to as differentiation.
Systems	The union of different organs forms the functional units that make up the macroscopic structures of the body with varied functions, for example the cardiovascular and nervous systems.
Mammalian body	A combination of different Systems that together comprise the complex body structure

Table 2. EDCs and Physiological systems.

Skin

The epidermis acts as a *protective layer* that impedes the entry of harmful chemicals. The vascular system does not extend past the dermis into the epidermis and this assists in allowing the skin to be a functional barrier to hazardous chemicals.

Blood vascular System

This is involved in *distributing* absorbed chemicals throughout the body.

The vast majority of the cells of the body are within 50 μm of a blood capillary which allows many chemicals to rapidly diffuse into the cell.

Chemicals ingested and absorbed from the digestive tract first enter the liver via portal circulation where they undergo several key biotransformations.

Chemicals that are inhaled can enter the blood vascular system via the pulmonary circulatory system without first being metabolized in the liver.

Respiratory System

This consists of a series organs and anatomical structures.

Since the nervous system and muscular system are involved in the mechanics of breathing many chemicals that affect nerve transmission adversely result in respiratory failure as a toxic response.

The inner lining of the functional surface where the exchange of respiratory gases occurs is very thin, in the region of 0.1-1 μm.

As a result inhaled chemicals come into close contact with blood allowing them to enter the *general circulation* without first being metabolized in the liver.

The Digestive System

Although a part of the digestive system it is generally accepted that the mouth does not significantly affect the absorption of hazardous chemicals. Most ingested chemicals are *absorbed* via the small intestine with a small proportion absorbed through the stomach.

Liver

This may be considered as a filter for blood in addition to numerous other physiological functions.

The liver contains specialized sinuses through which blood comes into contact with the functional liver cells where metabolism and key *biotransformations* of many chemicals occurs.

The liver can also inactivate hormones and transform other substances which are then transported via the circulatory system to the kidneys for excretion.

Enzymes present in the liver are crucial to numerous biochemical reactions pertaining to EDCs. These will be presented in more detail later.

Kidneys

The kidneys allow blood composition to be maintained by variable *reabsorption* of filtered blood and secretion of substances. Substances not reabsorbed or secreted may pass into the urine and be excreted.

2.5.2 The Endocrine System

2.5.2.1 General Aspects of the Endocrine System

The endocrine system is not confined to a particular organ but rather consists of a system in which varied processes and functions are regulated, both in the short-and long-term. These processes and functions include:

- general metabolic processes
- nutrition
- behaviour
- reproduction
- growth
- digestive tract
- kidney
- cardiovascular
- immune

The term “endocrine” has predominantly been used in reference to ductless glands that secrete hormones into the blood. A hormone is a chemical substance that is synthesized and secreted by ductless endocrine glands directly into the blood vascular system. Once in the circulation they are transported through the body to another target, typically a specific cellular receptor, where they may have an effect on specific biochemical processes.

The classic list of endocrine glands included those which exist as discrete organs, but now includes those found in association with exocrine glands and those found within complex organs (pituitary, thyroid, parathyroid, adrenal, and pancreas, ovary, testis, kidney, brain, gastrointestinal tract, placenta and pineal gland). Many organs are now also recognized as having an endocrine function, like the kidney for example.

The concept of limiting the endocrine system to instances where a hormone is secreted by a ductless gland has changed somewhat as new chemical regulators have been discovered. The term “neurohormones” is used to refer to the secretion of chemicals into the blood by neurons. The term “cytocrine” is used where local and intercellular chemical regulators are involved. Where intercellular cytocrines are transported to other cells in a tissue via the extracellular fluid the terms paracrine (affect other cells) and autocrine (affects themselves) are used. The term “intracrine” is used for intracellular regulators which act as second messengers and transcription factors.

The GWRC [10] presents a recent concise introduction to the endocrine systems relevant to EDCs that is sourced from several publications. This review presents primarily on the topic of steroid hormones (androgens, progestagens, glucocorticoids and mineralocorticoids), with a previous GWRC report in 2008 [12] focusing on oestrogens.

It should be noted that recognition and research into numerous other hormones affected by EDCs is noted in the literature, but it is beyond the scope of this Manual to address all the complexities of the endocrine system and associated EDC research topics. The additional hormones include those involved in calcium homeostasis, polypeptides (e.g. growth hormone), catecholamines, pancreatic metabolism regulators (insulin and glucagon) and hormones affecting kidney function.

The general mode of action and some key selected subsystems, or "axes" are highlighted, primarily as the current Volumes offer methods to investigate their effects. The main pathways of hypothalamic-pituitary-gonadal (HPG), hypothalamic-pituitary-adrenal (HPA), and hypothalamic-pituitary-thyroid (HPT) are thus referred to. An example of some of the complexities is presented for the reproductive and thyroid systems next.

2.5.2.2 Endocrine Aspects of the Reproductive System

Many aspects of reproduction are mediated by a combination of nervous and endocrine systems, referred to as neuroendocrine control. This control system is located in the hypothalamus and pituitary glands (present in the brain). The hypothalamus is situated "on-top" of the pituitary and the two are connected by a stalk containing neurosecretory fibers and a complex blood vessel arrangement. The pituitary gland may be further divided into an anterior portion (adenohypophysis) and a posterior portion (neurohypophysis). These portions in turn secrete different hormones in response to the hypothalamus which may act directly on target tissues or in turn control the secretion of other endocrine glands.

The secretory products of endocrine glands involved in reproduction are referred to as Reproductive Hormones and may be further divided into different classes.

Some Chemical Classes of Reproductive Hormones:

- Peptide and Protein Hormones:
  - Releasing Hormones:
    These are produced in the hypothalamus and are transferred to the pituitary gland where they regulate adenohypophyseal hormones. An example is GnRH (Table 3).
  - Hypophyseal Hormones:
    The releasing hormones are important in regulating the synthesis and/or release of these hormones. Examples include luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the adenohypohysis.
  - Neurohypophyseal Hormones:

The posterior portion of the pituitary gland stores and releases some hormones that are actually synthesized in the hypothalamus from which they are transported to be stored until neural stimuli cause their release into the bloodstream. Oxytocin is an example of a reproductive neurohypophyseal hormone.

- Steroid Hormones:
  The common precursor for these hormones is cholesterol. Over 1500 biologically active steroids have been isolated from biological material..
- Prostaglandins:
  These are unsaturated fatty acids and although not hormones in the classic context (not secreted from any particular gland and has predominantly a local action) the play an important role in regulating reproductive processes.

Interactions between these classes add to the complexity of reproductive processes. As an example, ovaries respond to gonadotropin by secreting oestrogen from follicles. Oestrogens initiate a release of LH and FSH through the release of GnRH and are linked to ovulation. Significant differences occur between mammalian species, further complicating the matter (e.g. differences in follicular and luteal phase overlap).

The hypothalamic and pituitary hormones exert their effects by binding to target cell surface membrane receptors with high specificity and affinity. For example, FSH released from the anterior pituitary affects cAMP in target gonadal tissue where a principal function is the stimulation of gametogenesis and follicular development in women and spermatogenesis in men. Both FSH and LH are also needed for proper reproductive functioning. Whilst LH stimulates androgen production FSH stimulates the conversion of androgen into oestrogens.

The field of pharmacology uses preparations of these hormones for therapeutic use. For example, an ampule of Menotropins contains 150 IU of FSH and 150 IU of LH, and may be administered for several days to women for the treatment of hypothalamic hypogonadism and for in vitro fertilization.

For diagrammatic representations of the endocrine control physiological and biochemical texts may be consulted [13], with appropriate EDC issues noted in specific reviews [10].

Table 3. Hypothalamic, pituitary and target gland hormones [14].

Hypothalamic Hormone	Pituitary Hormone	Target Organ	Target Organ Hormone
Growth Hormone-releasing hormone (GHRH)	Growth Hormone (GH)	Liver	Insulin-like growth factors (IGF)
Somatotropin release-inhibiting hormone (SRIH)			
Corticotropin-releasing hormone (CRH)	Adrenocorticotropin (ACTH)	Adrenal cortex	Glucocorticoids, mineralocorticoids Androgens
Thyrotropin-releasing hormone (TRH)	Thyroid-stimulating Homone (TSH)	Thyroid	Thyroxine, triiodothyronine
Gonadotropin-releasing hormone (GnRH)	Follicle-stimulating hormone (FSH)	Gonads	Oestrogen, progesterone, testosterone
	Luteinizing hormone (LH)		
Dopamine	Prolactin (PRL)	Breast	-

2.5.2.3 Endocrine Aspects of Thyroid Function

Extensive publications exist on disorders involving the thyroid gland in humans making thyroid function possibly the most commonly reported endocrine disorder. Nutritional disorders involving iodine and thyroid function are also well documented. An anatomical consideration regarding thyroid function relates to the fact that it is a highly vascularized tissue. Although the functional unit is considered to be the thyroid follicle (the source of thyroid hormones), interspersed are also thyroid C cells which produce the hypocalcemic hormone Calcitonin. Parathyroid glands are often considered as a third type of thyroid tissue as they may be imbedded in the thyroid gland in some species (or located in close proximity) and produce parathormone (a hypercalcemic hormone).

What makes the thyroid gland so relevant to water quality is that iodine is an intergral part of its hormone, thyroxine (T4). Selenium is also required for the activation of iodine for its inclusion into T4.

Since iodine is required the occurrence of iodine-deficiency disorders is well-documented. In addition the thyroid gland is different to most endocrine glands in that it is capable of storing large quantities of hormone with the iodine content a significant proportion of its weight. The principal hormones elaborated by the thyroid are T4 (3,5,3',5'-tetraiodothyronine), T3 (3, 5, 3'-triiodothyronine), and rT3 (reverse T3), with T3 being the active form in the target cell and T4 the transport form and feedback regulator.

The compensatory hypertrophy of thyroid follicular cells is well-documented and results in the development of iodine deficiency goiters (enlargements of the thyroid gland). Some chemicals may be disruptive by blocking the thyroid trapping of iodine (which is normally stimulated by TSH). Thyroid hormone has numerous effects, including clinical, developmental to reproductive, and as such is involved in numerous metabolic processes. Goiter is regarded as an enlargement of the thyroid gland that is not due to inflammation or malignancy and may be divided into nontoxic (simple or hypothyroid) and toxic (hyperthyroid). Deficiency or defect at any of the trophic steps may result in thyroid disease, with goitrogenic chemicals blocking the hormonogenic pathways.

Complications in the diagnosis of thyroid dysfunction are the ability of the gland to undergo hypertrophy and hyperplasia to increase uptake and secretion so that normal hormone levels are maintained despite visible increases in thyroid mass.

2.5.2.4 Goal of the Endocrine System

The goal of the endocrine system is to allow for dynamic coordinated responses in a target tissue to occur due to signals that originate in a different organ somewhere else in the body. This allows for the body to maintain homeostasis in which target cells are not only affected by the endocrine secretion but also affect the secretion itself via feedback mechanisms.

This homeostasis may be thought of as a mechanism for reducing the degree of change that ultimately affects metabolic processes, for example minimizing the concentration changes of blood glucose following a meal.

Understandably the endocrine system involves more than one pathway, for example, reproduction is influenced by age, nutrition and environmental stimuli. The endocrine system also maintains changes required to deal with changing physiological status, such as lactation and pregnancy.

In their scientific statement The Endocrine Society [8] recognizes the link between endocrine disruption and homeostasis by stating that: "An endocrine-disrupting substance is….alters the hormonal or homeostatic systems…"

It follows that endocrine disorders may result in significant AEs. Due to the interrelationships between the endocrine systems disorder in one system may thus also significantly affect other endocrine

systems and potentially involve multiple organs. It is largely due to this potential that EDCs are viewed as a serious threat to human and animal health.

This general perspective held also extends to the fact that disruption following exposure may not necessarily cause dysfunction. This implies that exposure to EDCs that may result in measurable disruption does not have to lead to a clinical manifestation of disturbance of the relevant system involved. The multiple factors that determine the outcome are presented next.

2.6 Key Concepts Relating to EDCs and Physiology

2.6.1 Key Events

Some of the key physiological aspects pertaining to exposure to EDCs are the events of absorption, metabolism and excretion.

EDCs & Physiology	
For EDCs to have an effect they must be:	***Absorbed***
Once absorbed EDCs are then subjected to various forms of:	***Metabolism***
Following this they may either be:	***stored*** or ***excreted***

The stages of absorption, metabolism and storage or excretion may be expanded to include:

routes of exposure and absorption
distribution through the body
biochemical transformation
chemical-receptor interactions
storage
excretion

These aspects are dealt with individually in the following section.

2.6.2 Routes of Exposure and Absorption

The type of effect that an EDC can have is influenced by the exposure route (place where it is absorbed). Some EDCs have adverse effects when inhaled, whilst others have more pronounced effects when ingested.

Two main absorption mechanisms are:

- passive transport (diffusion) or active transport into cells
- movement between cells (pores or channels)

Properties of EDCs that influence passive transport include:

- degree of ionization, lipid solubility, protein binding and water solubility

EDCs with high lipid solubility tend to have high rates of diffusion through membranes.

The routes of exposure and relevant aspects that are applicable include:

Dermal	As the skin serves as a physical barrier dermal exposure is usually a minor route. Some chemicals can however be absorbed through the skin (e.g. parathion) and these are typically nonpolar compounds (metallic mercury having polarity is not readily absorbed through the skin whereas dimethyl mercury which is more nonpolar is). Most direct contact occurs in industrial settings (e.g. organic solvents).
Inhalation	This is the main route of exposure for airborne compounds, gasses and volatile EDCs. The main mechanism of absorption for inhaled substances is diffusion. Once the substance dissolves in the liquid layer of the alveoli (function units of gas exchange in the lungs) they can be removed by the pulmonary blood system. They may then be distributed to cells prior to undergoing metabolism in the liver.
Oral	EDCs often enter the body in the same manner as nutrients, namely through the digestive tract. Here contaminated drinking water is the most common route, but contaminated food and soil are also potential mechanisms. Some protection is afforded by poor absorption from the digestive tract and by interference with other ingested substances and products of digestive processes. Once absorbed the substances are first exposed to variable metabolic processes in the liver.

2.6.3 Distribution and Storage

The vascular system transports EDCs once absorbed throughout the body via a portion of the cardiovascular system referred to as systemic circulation. Most absorbed substances do not have a localized effect at the point of absorption, but rather at different places in the body where they have been transported to and subsequently attach to receptor biomolecules. Organs where binding occurs (and hence where adverse effects may occur) include the liver, kidneys, reproductive organs and the blood itself.

Storage does not necessarily occur. Storage consequences of EDCs binding to different biomolecules are referred to as partitioning behaviour. Products that are highly water soluble tend to not partition into fats and are thus generally rapidly eliminated via the urine. Organic chemicals have high lipid solubility and tend to be stored in fat where they can remain for years. Some metals can also be stored by binding to fat negatively charged sulphur-containing groups of proteins. These can occur in different organs and systems. Lead, for example, binds to the nervous system whereas cadmium tends to bind to the kidney.

2.6.4 Biochemical Transformations

Biochemical transformations, or biotransformations, are the primary fate of absorbed chemicals with most resulting in products that have no adverse effects. When the products yield toxic metabolites the process is termed bioactivation. This process provides the basis for most toxic and carcinogenic (cancer-forming) effects following absorption of hazardous chemicals. Some key enzymes that catalyze these bioactivation reactions are presented in Table 4.

Table 4. Key Bioactivation reactions.

Phase 1 biotransformations	Transforms hydrophobic chemicals to more polar products. Includes processes such as oxidation, hydrolysis, reductions, deaminations, dehalogenation, sulphoxidations and conjugations. Typically a hydroxyl group is added to a hydrophobic compound to increase water solubility. The catalyst enzymes are nonspecific (mixed function oxidase or MFO, cytochrome P-450 system, or the cytochrome P-450 dependent monooxygenase system). Products may or may not cause adverse effects.
Phase II biotransformations	These are conjugation reactions that add polar functional groups (e.g. glucose and sulphate) to the Phase I products resulting in products that are more polar and can be readily excreted. A reactive functional group (e.g. -OH) binds to a cosubtrate (e.g. glucose) producing a product that has a higher degree of water solubility and hence easier excretion via the urine or bile. The product, by virtue of its structure, can also have reduced binding to some receptors which decreases potential adverse effects.
Phase I and Phase II are complementary, with Phase I adding functional groups that are required for Phase II biosynthesis reactions. Many enzymes catalyze these biotransformation reactions and many have broad substrate specificity that enables them to transform a wide range of hazardous compounds into products that can be easily excreted. Most of the biotransformation reactions occur in the liver, but significant reactions also occur in the kidneys and lungs, with some also taking place in the digestive tract and reproductive organs.	
Some ionized water-soluble inorganic compounds (e.g. common table salt or sodium chloride or NaCl) are readily excreted and often do not require any biotransformation. Some polar organic compounds (e.g. ethyl alcohol) are readily excreted and often do not require any biotransformation. As a result, the extent to which an EDC undergoes biotransformations is often correlated to the hydrophobicity of the EDC. Thus hydrophobic compounds tend to require biotransformations before being effectively excreted by the kidneys. Some hydrophobic compounds can be directly excreted through the bile system of the liver.	
Mammals have highly variable biotransformations that are specific to the enzymes present, the tissue type and organ development of the species, resulting in significantly different responses to the same EDC between different species.	

2.6.5 Receptor-Chemical Interactions

This is a central topic with reference to endocrine disruption. Concern exists for the potential of hazardous effects from exposure to EDCs in living organisms. Implicit in this concern is the central concept that the organism acts as a receptor.

Enzymes are the most common types of receptors involved, but other receptors are also affected, such as hemoglobin, DNA and membranes. Many toxicological terms stem from the organ or tissue type affected by this binding, for example, hepatoxicity refers to the liver, genotoxicity to the chromosomes.

The concept of structural affinity is often referred to as the key driving force behind a hazardous effect as it suggests a potentially hazardous chemical can have an adverse effect by binding to a specific receptor in a lock and key fashion. Once bound some or all of the functions of the receptor may be adversely affected. An example of this is the binding of carbon monoxide to hemoglobin that displaces oxygen and results in asphyxiation. Quantitative structure-activity relationships (QSARs) are used to investigate the links between chemical structure and receptor molecules.

In order to understand receptor-chemical interactions the following issues need to be discussed:

Toxicant

A toxicant, poison or harmful chemical is defined as any solid, liquid, or gas that interferes with life processes, ranging from the molecular level, to the population level [1].

Drug

A drug may be defined as any substance that brings about a change in biological function through it chemical actions. The vast majority of these actions involve a drug molecule interacting with a specific molecule in the biologic system with a regulatory function, referred to as a receptor molecule [14].

Requirements:

A receptor can only interact on a chemical basis with an EDC if the EDC has the appropriate shape, size, electrical charge, and atomic composition.
Transport from site of exposure to the receptor must also occur.
The actions must also occur to a significant extent prior to excretion.

Reactivity and EDC-receptor bonds:

The interactions occur due to chemical forces or bonds which are covalent (very strong and often irreversible), electrostatic (vary from strong links between charged molecules to weaker hydrogen bonds and even weaker dipole interactions) and hydrophobic (usually weak and involve lipid-soluble EDCs and cell membrane lipids)

As a general rule EDCs that bind to receptors with weak forces are usually more selective than those that bind with strong forces.

Receptor shape:

Enzymes are usually stereoselective. The phenomenon of chirality (stereoisomerism) is common in biology implies that in most cases one enantiomeric molecular pair of an EDC can have a better "fit" to the receptor molecule. If the receptor site is thought of as a left-handed glove, then an EDC molecule that is "left-orientated" will "fit" better than a "right-oriented" enantiomer. In most cases EDCs can be thought of as mixtures of these shapes making studies on clinical effects based on dose-responses difficult to interpret.

Receptor-EDC interaction types:

These can be thought of a two separate types of processes. One in which the EDC has an effect on the body (dynamics) and the other in which the body has an effect or action on the EDC (kinetics). Kinetics thus involve absorption, distribution and excretion, whereas dynamics require the EDC to bind to a receptor to have an effect.

Dynamics:

Different types of effects can arise from EDC-receptor binding.
Although there are many variations the main types are:

Agonistic: the EDC binds to the receptor and activates it to cause some effect.
Antagonistic: the EDC binds to the receptor and prevents it from binding to other molecules

Kinetics:

These aspects of absorption, distribution and excretion were dealt with earlier.

Consideration of types of effects:

The various types of effects considered when assessing hazards and risks from exposure to potentially hazardous chemicals are presented in Table 5.

Table 5. Fundamental concepts of hazardous effects.

Toxicity	Although some direct damage to cells can occur (localized toxicity) this is not common. An example of this would be direct contact of the skin with a strong acid or base. The primary mechanism in which a chemical can result in an adverse effect involves binding to receptor molecules of a cell, tissue or an organ. An example of this is parathion inhibition of acetyl cholinesterase resulting in neurotoxicity. May also be expressed on a macroscopic level, for example tissue necrosis (cell death due to chemical injury).
Carcinogenicity	This refers to altered cells that divide in an uncontrolled manner (neoplastic growth). This leads to the development of tumors (neoplasms) that can be benign (inert masses) or malignant (proliferate with adverse consequences). The long period between exposure to a hazardous chemical and the expression of cancer (latency) complicates the description of the chemical-receptor relationship. Types of cancers often refer to the system they affect, for example, sarcomas refer to connective tissue cancers.
Endocrine Disruption	Altered function(s) of the endocrine system causing (potential) adverse health effects in an intact organism, or its progeny, or (sub)populations.

2.6.6 Exposure Considerations

Whilst the type of EDC involved in an exposure is critical, as a general statement the conditions around the exposure that are independent of the EDC involved are also crucial as to whether an AE will result. Changes in the level, duration and timing of the exposure are all factors that can result in different outcomes following exposure to the same EDC.

It is generally regarded that the following considerations are critical:

- Exposure during adulthood may be less sensitive than exposure during developmental stages.
- Exposure occurring during embryological and developmental stages may result in permanent damage. This may manifest at a later stage after exposure has occurred or ceased.
- Exposure may result in AEs in more than one endocrine system, and a primary effect on one endocrine system or organ may manifest in a different system.

These factors contribute to the uncertainty in predicting the outcomes following EDC exposure. This may also make it difficult to repeat observations of specific outcomes across different communities despite having exposure to the same EDCs. It is therefore understandable that a precautionary

approach is followed and extrapolation from *in vitro* measure of endocrine disruption to actual exposure scenarios (*in vivo*) should be made cautiously.

2.7 Current EDC Overview

Numerous organizations, scientific societies and public interest groups have published opinions and fact-sheets on EDCs. The reader is referred to the Selected Reference list for further detailed reading. This section touches on some of the changing perceptions and constant themes observed in these statements and research focus.

It is best noted upfront that endocrine disruption does not fit in the classic definition of toxicity and is thus not in itself a toxicological end point. Endocrine disruption may however be viewed as a functional change that may lead to adverse effects. As this field is new and continuing scientific investigations shed new light on different types of endocrine disruption it is generally considered prudent to adopt a precautionary principle.

As noted in the introduction section 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.

A potential endocrine disruptor is:

- an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny or (sub)populations.

EDCs have been demonstrated to act at more than one site via multiple mechanisms. Although receptor-mediated mechanisms have received most of the research attention, other mechanisms are increasingly implicated, such as the actual synthesis, transport and metabolism of hormones [9; 11].

In many instances the actual mechanism involved in producing the observed disruption is not entirely understood and as such distinguishing between primary and secondary effects (and direct and indirect effects) is difficult.
Although it is therefore argued that this requires a cautious approach when extrapolating from *in vitro* data to *in vivo* effects, similar cautions apply when extrapolating experimental data collected from animals to human situations. These extrapolations are a fundamental basis of risk assessment and dealt with in more detail under the Monitoring and Assessment section.

What is generally agreed upon is that due to the potential long-term, developmental and irreversible nature of the adverse effects a precautionary approach should be taken. This is referred to as the

Precautionary Principle [5] and often based on the significant amount of current evidence of AEs pertaining to the reproductive system and growing evidence of same in other systems.

Numerous differences may occur with regard to the types of effects following exposure to an EDC. As presented earlier the route of exposure can alter the manner in which the EDC is handled by the body, both in terms of absorption and metabolism. Differences in exposure duration can also result in different reactions. Generally both acute (1-2 days) and chronic exposures are relevant to EDCs in water.

What is particularly challenging is the observation of effects following exposure to low concentrations over long periods of time, referred to as low-level, long-term exposure. Effects may also be shortly after exposure or after a significant interval (delayed). Repetitive exposure over set periods may also only present with AEs once recuperative properties of the system or organ are exceeded.

Some relevant issues in linking EDCs to AEs include:

- Time of exposure:
  - If exposure occurs during the developmental stage of the endocrine system the consequent effects may result in a permanent change or loss of function or sensitivity.
  - If exposure occurs in adulthood normal homeostatic mechanisms may be sufficient to compensate for the effects with no significant or clinical adverse effects observable.
- Context of exposure:
  - The same degree of exposure may have different effects in individuals with different histories or different seasonal exposures.
- Due to feedback mechanisms and numerous links between the components of the endocrine system exposure may result in unpredictable effects both in terms of the target tissues and type of adverse effect.
- Due to the complexities between the large number of potential EDCs an organism can be exposed the outcomes of concurrent exposure to multiple EDCs are very difficult to predict. Effects may range from additive, supra-additive (synergistic) to infra-additive (antagonistic). Potentiation may also occur where effects are only noted when specific combinations of EDCs are present in the exposure mixture. Failing evidence to the contrary it is normally assumed the effects are synergistic.

Since EDCs can mimic or antagonize the actions of naturally occurring hormones it is challenging to demonstrate clear dose-response relationships. This relationship is a fundamental component of describing concentration-effect curves and receptor binding used both for toxicology and pharmacology. In these fields of study it is well documented that carefully controlled *in vitro* systems can yield precise relationships between concentration and effect, yet more complex relationships between dose and effect occur in patients or exposed populations.

It is usual to observe an increase in the response to low doses of a chemical in direct proportion to increases in the dose. At higher doses this response tends to diminish to a point where no further increase is noted with higher doses. This is typically represented by a hyperbolic curve and allows for the calculation of the maximal response that can be produced by the chemical. This curve suggests (as radioactive receptor ligands have proven) that a drug agonist acts by binding to a distinct molecule with affinity for the drug receptor.

The graphic representation of dose-response data is often presented by plotting the drug effect (ordinate) against the logarithm of the dose or concentration (abscissa), and in so-doing transform the hyperbolic curve into a sigmoid one. The benefit of this is that the mid-portion is more linear and allows for easier comparison between dose-response curves as the concentration axis scale at low concentrations is expanded whilst the area of high concentrations are compressed. The reason this helps is that most of the rapid changes in response occur at low concentrations whilst less noticeable effects occur at high concentrations.

Once a chemical has bound to a receptor the conformation change allows a transduction process between occupancy of the receptor and response, termed coupling. Variations in the conformational change may occur as a result of different efficiencies of occupancy-response coupling and lead to terms such as full agonist and partial agonist. The resultant biochemical events that transduce receptor occupancy into cellular response may determine the coupling efficiency. The concept of "spare" receptors is used to explain how a maximal response may still be elicited by an agonist even when the full complement of available receptors is not occupied by it, either because the agonist concentration is too low or irreversible antagonists are present. This is demonstrated in cardiac muscle where maximal response to catecholamines can be achieved even though 90% of the beta receptors are occupied by an antagonist. This biochemical mechanism behind many types of "spare" receptors is not well understood.

Receptor antagonists bind to the receptor without resulting in activation and may be competitive or irreversible. The use of antagonists is well illustrated in cardiac pharmacology and highlight similar challenges to describing EDC exposure and AEs. For example, the use of a beta antagonist in a patient to maintain a desired heart rate must be considered in conjunction with the variability in endogenous agonist concentration so that the use of the competitive beta-adrenoceptor drug must be at a sufficient dosage to not only block the basal levels of norephinephrine when at rest but also with exercise or emotional stress that can result in increased norepinephrine and epinephrine levels that could potentially overcome the competitive antagonism and result in potentially adverse increases in heart rate.

It is important to note that antagonism need not have to be preceded by interactions with a receptor. A chemical can antagonize the actions of a substance by binding to and in so-doing inactivate it (prevent it from binding to a receptor). The use of chemical antagonists is common in the clinical

environment where physiologic antagonism is used to obtain a desired endogenous regulation. The use of insulin to oppose the hyperglycemic effects of glucocorticoid hormone is an example of this.

When compared to standard environmental toxicological studies on hazardous effects from exposure to specific chemicals the presence of endogenous hormones that may be more potent than exogenous EDCs result in different dose-response considerations for EDC research. It has been argued that EDC effects are possible at such low exposure concentrations that the standard approaches fail to note the effects [9]. These dose-response relationships also vary with the type of EDC in question.

Although in many cases clear cause and effect relationships have yet to be comprehensively described the concern of adverse effects in humans (mainly reproductive and developmental) linked to EDC exposure is accepted as biologically plausible when the known influences of background endogenous and exogenous hormones on these processes are considered. A brief summary of reported types of effects are presented in Tables 6 and 7.

It is furthermore generally stated that the epidemiological evidence of trends in certain diseases and disorders are sufficient to warrant concern and accord a high research priority to EDCs and potential AEs.

In an attempt to create an objective and unbiased assessment of the hypothesis that EDCs have AEs in the experimental context, humans, and wildlife, in their Global Assessment of EDCs the IPCS [9] considered a framework for examining firstly the hypothesis and then the trends. Whilst acknowledging that scientific uncertainties exist and a degree of scientific judgment or expert opinion is involved, and that the assessments will change over time as new information becomes available, the approach not only provides an assessment but in doing so helps with the identification of knowledge gaps. The hypothesis had two distinct elements, firstly it considered the outcome of concern linked to the putative stressor, and secondly, the endocrine-mediated events following exposure to the stressor. Five specific aspects were considered: temporality, strength of association, consistency of observations, plausibility of the effect, and evidence of recovery following stressor diminution. Some illustrative examples of this proposed framework for assessing EDCs are provided in Tables 8 and 9.

Table 6. Summary of EDC effects reported in wildlife [9].

Exposure Subject	**Types of Effects following exposure to EDCs**	
Wildlife	Generally effects observed have been able to link EDCs to adverse effects. These vary from subtle physiological changes to changes in sexual behaviour and permanent altered sexual differentiation. Species most affected are aquatic species (top of food chain). Causal link between exposure and endocrine disruption often unclear. Suggested use of wildlife as sentinels for human exposure not without extrapolation difficulties. Most well-described effects related to high exposure concentrations.	
	Examples:	
	EDC Type:	**AE:**
	Organochlorines (PCBs, DDE)	Adverse effect on reproductive and immune function in Baltic seals leading to significant population decline.
	DDT	Eggshell thinning and altered gonadal development in birds of prey leading to significant population declines.
	PCB	Embryonic abnormalities in fish-eating birds.
	Chemical spill of organochlorines	Gonadal and developmental abnormalities in Alligators.
	Varied	Population decline in amphibians but with insufficient causal data.
	Industrial effluent from pulp and paper mill and sewage effluent	Extensive evidence of altered reproductive development in fish with numerous mechanisms involved (hormone-receptor interactions, interference with hormone synthesis, altered endocrine gland function).
	Biocide (TBT)	Invertebrates (marine gastropods) demonstrate masculinization leading to population decline worldwide.

Table 7 Summary of EDC effects reported in humans [9].

Exposure Subject	Types of Effects following exposure to EDCs	
Humans	Currently general population adverse effects not clearly demonstrated by firm evidence of direct causal associations between low-level exposure to EDCs and AEs. Typically insufficient or incomplete data collection, specifically on exposures. In many cases the link between EDC exposure and the observed AE remains speculative and the detail of the mechanism largely unknown. Despite high endogenous concentrations and potencies and the presence of phytoestrogens at higher concentrations than most EDCs, concern still exists for role of EDCs in adverse health outcomes.	
	Examples:	
	AE: Reproductive	Numerous reports on sperm quality decline. High-level exposure to certain chemicals demonstrated to impair fertility and increase rate of spontaneous abortion. Declining sex ratios (fewer males) recorded. Temporal increases in frequency of developmental abnormalities in male reproductive tract, notably cryptorchidism and hypospadias. Experimental evidence has demonstrated endocrine mechanisms to be involved in developmental disruption of the male reproductive tract due to chemical exposure.
	AE: Endometriosis & Precocious puberty	Both claimed to be associated to EDC exposure but studies remain equivocal.
	AE: Neural function	Human and experimental animal studies clearly indicate exposure to some EDCs (PCBs) to have resulted in AE on neurological development, neuroendocrine function, and behaviour. Specifically prenatal exposure a sensitive exposure time. Thyroid and neurotransmitter function involved.
	AE: Immune function	Some chemicals have been shown to alter immune function, however, exact endocrine-mediated pathway unclear.
	AE: Cancer	Often cited as evidence of EDCs AE on general population, specifically in hormonally sensitive tissues in populations in parts of the industrialized world. Difficulty in differentiating between increased release of chemicals and specific endocrine-mediated pathways as causative effects.
	AE: Breast Cancer	Although still contentious numerous studies appear to indicate a link between EDCs and the incidence of breast cancer. Data sets lack observations on critical developmental periods (most measure EDC exposure in adult women).
	AE: Testicular Cancer	Concerns relate to temporal increases in testicular cancer. Similar concerns exist for cryptorchidism and hypospadias with regard to geographical variations of testicular cancer. Developmental route suspected, but critical exposure period data lacking.
	AE: Prostate Cancer	Specific pesticides and organochlorines suspected as a link.
	AE: Thyroid Cancer	Although a clear direct link has not been demonstrated it is suspected.

Table 8. IPCS proposed framework for assessing EDCs [9].

Statement of Hypothesis		**Evaluation Factor**					**Strength of Evidence**	
Outcome	**Stressor**	**ST**	**A**	**BC**	**Pl**	**Rc**	**For Hypothesis**	**For EDC Mechanism**
Endometriosis in humans	TCDD, PCBs	**ND**	*	*	*	**ND**	**Weak**	**Moderate**
Impaired neurobehavioural development in humans	PCBs	****	***	***	***	**ND**	**Moderate**	**Moderate**
Perturbed immune function in humans	PCBs, TCDD	***	****	**	**	*	**Moderate**	**Weak**
Incidence of breast cancer in humans	DDT, DDE, PCBs	*	*	*	**	**ND**	**Weak**	**Weak**
Imposex in marine gastropods	TBT	****	****	****	***	****	**Strong**	**Strong**
Decreased reproductive function in Baltic seals	PCBs	***	**	***	***	****	**Strong**	**Moderate**
GLEMEDS in birds	PCBs	****	****	****	****	****	**Strong**	**Weak**
Egg shell thinning in colonial waterbirds	DDE and DDT metabolites	****	****	****	***	****	**Strong**	**Moderate**
Reproductive abnormalities in alligators	Dicofol and pesticides	****	***	***	***	**	**Moderate**	**Moderate**
Developmental abnormalities and reproductive failure in trout	Dioxins and coplanar PCBs	****	****	***	****	****	**Strong**	**Weak**
Vitellogenin induction in fish	Oestrogenic	****	****	***	****	**	**Strong**	**Strong**

Where:

ST = Strength of Temporality, A = Association, BC = Biological Consistency, Pl = Plausibility, Rc = Recovery.

ND = no relevant data

*** to **** = weak to strong**

Table 9. IPCS examples of status and trend evaluation of EDC data [9].

Statement of Hypothesis		**Evaluation Factor**					**Overall Strength of Hypothesis**		
Outcome	Stressor	T	SA	C	BP	R	For Outcome	For Hypothesis	For EDC mcm
Reduction in semen quality and testis function in humans	Oestrogenic and antiandrogenic chemicals	ND	ND for Asc * for effect	ND for exp * for effect	***	ND	Weak	ND	Weak
Limb mal-formations in frogs	Unknown chemical etiology	ND	ND for asc ** for effect	ND for exp * for effect	**	ND	Strong	Weak	Weak

Where:
T = Temporality, SA = strength of association, C = Consistency, BP = Biological Plausibility, R = Recovery,
ND = no relevant data, Asc = association, Exp = exposure, mcm = mechanism
*** to *** = weak to strong**

The reasons cited for assessing the trends as weak regarding semen quality and testis function in humans relate to deficits in the available data sets [3]. For example, whilst it was recognized that sperm count and semen volume have shown significant declines, none of the studies included prenatal, childhood, or adult exposure assessment for oestrogenic or antiandrogenic chemicals, and the decline supposedly began before the widespread use of industrial chemicals. The available data relating to cause-and-effect relationships and consistency of effect with specific chemicals is also largely unsuitable.

However, the human data for incidence trends in developmentally related end-points such as testicular cancer and male reproductive tract abnormalities is supportive of the plausibility of the biological processes involved with EDCs and the suspected AEs. This is furthermore supported by experimental observations in animals. In these studies, the periods lacking in human studies have been found to be particularly vulnerable (prenatal and perinatal).

Similar statements on deficits in available evidence are noted by the IPCS [3] and contribute to the comments that appear in Tables 6 to 9.

However, in one of the most prominent first releases on the topic from a scientific society several years later The Endocrine Society [5] presented evidence for endocrine disruptors having effects on male and female reproduction, breast development and cancer, prostate cancer, neuroendocrinology, thyroid, metabolism and obesity, and cardiovascular endocrinology, and observed that:

- Results from animal models, human clinical observations, and epidemiological studies converge to implicate EDCs as a significant concern to public health.

Thus, Tables 10 and 11 indicate increasing confidence in the possible mechanisms and significance of EDC exposure and AEs, and The Endocrine Society [5] presents many more examples of more comprehensive disorders and EDC pathways.

This led to the society invoking the precautionary principle and advocating increased involvement of individual and scientific society stakeholders in communicating and implementing changes through public policy and awareness.

Table 10. Examples of some EDC effects on the male reproductive system [8].

EDC	**Exposed animal and effects**	**Possible translation to clinical condition**	**Potential mechanisms**
Vinclozolin	Fetal rat: hypospadias; undescended testes, prepubertal; delayed puberty, prostate disease in progeny	-	Epigenetic; altered DNA methylation in germ cell lines.
DES	Fetal rats: hypospadias, cryptorchidism, micropenis, increased transmitted susceptibility to malignancies	Hypospadias, cryptorchidism, micropenis, epididymal cysts	Increased ERl expression in epididymis. Reduced insulin-like factor 3.
DDT	Adult rats: decreased fertility	Cryptorchidism	-
DDE	-	Cryptorchidism	-
Phthalates	Reduced anogenital distance. Cryptorchidism Oligospermia	Reduced anogenital distance and Leydig cell function, hypospadias. Cryptorchidism. Reduced fertility	Decreased testosterone synthesis
PCBs	Fetal rat: decreased spermatogenesis, delayed puberty	Reduced penile length, delayed sexual maturation, reduced fertility Fetal: testis cancer.	-
BPA	Increased prostate size. Aberrant development of prostate and urethra.	-	Increased ERl expression in hypothalamus. Increased AR expression

EDC	Exposed animal and effects	Possible translation to clinical condition	Potential mechanisms
	Prostate cancer Increased anogenital distance, altered periductal stroma in the prostate.		in prostate.

Table 11. Examples of some EDC effects on the female reproductive system [8].

EDC	Exposed animal and effects	Possible translation to clinical condition	Potential mechanisms
Vinclozolin	Fetal rat: multisystem disorders including tumors	-	Epigenetic; altered DNA methylation in germ cell line; reduced ERα expression in uterus.
DES	Fetal mouse: transmitted susceptibility to malignancies	Vaginal adenocarcinoma in daughters of women treated with DES during pregnancy	-
DDT/DDE	Immature female rat: sexual precocity	Precocious and early puberty. Reduced fertility in daughters of exposed women. <15 yr: increased risk of breast cancer	Neuroendocrine effect through oestrogen receptors, kainate receptors, and AhRs
BPA	Inhibited mammary duct development and increased branching. Increased mammary gland density, increased number of terminal ends. Reduced weight of vagina. Endometrial stimulation	Miscarriages	Inhibition of apoptotic activity in breast. Increased number of progesterone receptor-positive epithelial cells. Reduced sulfotransferase inactivation of estradiol. Nongenomic activation of ERK1/2
PCBs	Fetal and early postnatal rat: Neuroendocrine effects in two generations, and behavioral changes.		Actions on oestrogen receptors, neurotransmitter receptors.
Dioxins	Fetal rat: altered breast development and increased susceptibility for mammary cancer. Early pubertal rat: blocked ovulation		Inhibition of cyclooxygenase2 via AhR.
Phthalates		Premature thelarche	

Despite the comprehensive evidence and descriptions of the molecular events following hormone response to receptor binding, by comparison the relationship between these events and AEs such as cancer and the reproductive problems mentioned is poorly described. This is often referred to as the knowledge gap in EDC exposure-response relationships.

Although numerous research fields are working to address this issue it is also now recognized that EDCs may interact with other receptor systems (orphan receptors) that also affect the endocrine system. Thus, AEs from EDCs are not limited to interactions with hormone receptors, but include mechanisms that inhibit hormone synthesis, transport, metabolism and activation of receptors and biochemical events required for hormone action to occur.

This recognition also relates to the reality that multiple receptor systems regulate biological functions. These are sometimes referred to as “cross-talk”, and an example involves antiandrogen-mediated increases of endogenous oestrogen levels resulting from increased LH production.

A particularly challenging aspect of EDCs and AEs relates to dose-response relationships. The difficulty is understood when seen in context of normal physiological aspects of endocrine regulation with hormones already being present at functional concentrations in healthy individuals and EDCs acting by mimicking or antagonizing their actions. In addition, the challenge of observing low-dose effects has led to the toxicological significance being questioned as well as the lack of consistency of observing AEs. In this regard much controversy is apparent in choosing between toxic and mechanistic end points.

It is generally recognized that when exposure data obtained from more than one source is gathered the link between EDC exposure and AEs are more credible. This implies that toxicity, mechanistic, epidemiological and field studies are contribute to contextual understanding of EDCs in human and wildlife health, and as such collaborative studies are becoming more evident.

It follows that it cannot be expected to describe a single dose-response relationship for all effects and all endocrine disruption mechanisms, as EDCs can affect a multitude of actions. These may thus include oestrogenic, antioestrogenic, antiandrogenic, growth factor modulation, cytokine and thyroid modulation, hormone synthesis and metabolism.

In the recent GWRC review [10] the situation was generally still supportive of the comments noted in this section. It noted that:

- Chemicals in the environment have been conclusively linked to endocrine disruption in wildlife, leading to:
  - Ovotestis in fish
  - Imposex in gastropods

    - Shrunken penises in alligators
    - Eggshell thinning and embryonic abnormalities in birds of prey
    - Pseudo-hermaphroditism in polar bears
- Despite the overwhelming and conclusive evidence of endocrine disruption in wildlife, the evidence thereof in humans is less so.
- Exposure scenarios from industrial settings have shown that the human endocrine system can be disrupted by EDCs.
- A wide array of pollutants is routinely detected in human blood.
- Uncertainty regarding the physiological significance of these concentrations detected still exists.

Despite this, the potential for EDCs at low levels of exposure to represent a serious threat to human life is generally accepted [10].

Within all EDC exposure scenarios the contribution of water must not only be investigated, but the perspective thereof to the total exposure should be placed in context.

In this regard there is much more research required. As noted in the GWRC review [10]:

- A meta-analysis should still be performed for assays investigating androgenic and progestagenic activity, and these have still not focused on water sample testing.
- Steriodogenesis interference as an endpoint still requires some basic science before measurements thereof can be meaningfully applied to water testing.
- The relevance of thyroid assays to water testing is still unclear.
- Despite the recognition of glucocorticoid, retinoid X receptor and peroxisome proliferator receptor as priority endocrine endpoints, little is known about their activity in water.
- Most non-oestrogenic assays have not been applied in a comprehensive manner to wastewater, with a lack of information on their activity in other waters, including drinking, surface and groundwater.

Given the reliance on groundwater in South Africa establishing the contribution and physiological significance of EDCs therein is viewed as a priority.

2.8 What Types EDCs are Known or Suspected?

Endocrine disruptors currently identified belong to a highly heterogeneous group including synthetic chemicals (industrial chemicals) and their byproducts (PCBs, PBBs, dioxins, plastics [BPA], plasticizers [phthalates], pesticides [DDT], fungicides [vinclozolin] and pharmaceutical agents [DES]). Since EDCs may be organic or inorganic chemicals, some exposure scenarios include naturally occurring EDCs.

EDCs also include a wide range of types of chemicals, from synthetic hormones to natural hormones. Chemicals found in human and animal food (phytoestrogens) may also be EDCs. Exposure may be to plant constituents, pesticides, industrial waste compounds and other pollutants. Some consumer products may also contain EDCs.

Due to the heterogeneous nature of reported and suspected EDCs it is difficult to predict whether a compound will exert endocrine-disruptive effects or not. However, some broad aspects often apply. For instance, dioxins, PCBs, PBBs and pesticides tend to contain halogen group substitutions by chlorine and bromine, and often have a phenolic moiety that is suspected of mimicking natural steroid hormones thus enabling them to interact with these hormone receptors as analogs or antagonists.

Metals and metalloids are also recognized as having the ability to be oestrogenic and androgenic, to act as antiandrogens, and as thyroid hormone receptor agonists or antagonists. This raises the important topic of generalized toxicants typically thought of as following a classic dose-response relationship and inducing toxic effects to now be capable of being an EDC as well.

Even an ubiquitous pollutant such as nitrate has been demonstrated to alter steroidogenesis and to disrupt thyroid function. Since nitrate and nitrite are wide-spread in environmental media as contaminants, notably water, the potential for it to be a significant EDC has seen it receive much research attention.

EDCs also range from chemicals that are persistent in the environment to those that degrade rapidly. These environmental considerations relate to the degradability, mobility and final bioaccumulation or biomagnifications of the chemical, and as such, the presence and occurrence of EDCs may vary greatly from one geographical site to another. This leads to a challenge in identifying EDCs and to draw conclusions regarding suspected EDCs and AEs due to fact that exposure-outcome relationships are hard to measure. Thus, some EDCs that may be involved in exposure during a critical period of development may still be of concern, even if they are not persistent and degrade rapidly. However, as these observations and measures are difficult, it is not surprising that many of the priority EDCs listed globally are biologically and ecologically persistent.

Twelve persistent organic pollutants (POPs) were identified in 2001 (Stockholm Treaty) [15] based on data that demonstrated AE based on exposure in humans and wildlife, and an undertaking was made by the 115 signatory countries to eliminate and/or reduce them. Most regulatory agencies throughout the world have two types of lists, one which enforces regulatory actions, and the second, which contains a list of suspected EDCs. Although these regulatory efforts have seen reductions in reported concentrations for some EDCs in some countries, the general trend is for both lists to grow.

It should be appreciated from the preceding discussion that a full review of the known and/or suspected EDCs is beyond the scope of this Volume, hence only some key examples are provided. Volumes 1 and 2 of the Manual should be consulted for further detail.

2.8.1 Types of EDCs

Although the list of substances considered as EDCs varies both locally and internationally a similarity in approach is generally observed in which they are considered as an on-going list of varying stages, from suspected EDC to a higher level of priority EDC. The adoption of the "precautionary approach" with regard EDC effects can result in the list of "suspected" EDCs to be somewhat larger than a list of substances dealing with clear toxic effects.

In order for a substance to be listed as an EDC it must first be detected in the exposure medium relevant which in this instance implies that it should involve water quality. This effectively covers the recognized water uses, for example, an EDC occurring in water is not only of concern if present in drinking water for domestic purposes, but also if water is used for irrigation of edible crops and the irrigation either results in the presence of an EDC in the edible product or contaminates the environment in some manner. A similar approach applies to irrigation of pastures for animal production and possible effects on not only animal health but additional norms for fitness for use such as product quality (e.g. milk quality).

The EDC must then also be able to be determined analytical at relevant concentrations and based on a range of different observations and/or experiments be linked to an EDC effect. A critical distinction here is that the link between concentration (presence) and endocrine disruption allows for the listing thereof as an EDC but does not require the link to be described to the same extent as for classic toxicity in terms of an adverse effect. This is a fundamental approach and challenge to EDC research and is largely influenced by the complexities of endocrine function with regards disruption, disturbance and physiological homeostatic processes.

As it is beyond the scope of this volume to present all the known and suspected EDCs an example of the complexities with regards to considerations for animal health and EDCs is briefly presented. This is done as evidence of significant endocrine disruption has been more detailed for animal exposures than for human exposures [10].

Three broad categories of EDCs may be considered to apply for animal health. The first consists of chemicals considered to be inorganic minerals that are both naturally occurring, supplemented in the production of forages used for animal diets, pastures and supplemented directly via dietary (total mixed rations) and other supplementary feed inclusions (feed premixes, licks, water treatments and injectable preparations). The second group consists of chemicals used that are generally classified as pesticides, herbicides, insecticides, disinfectants and fungicides. In order to maintain the recognition of complexity for differential diagnostics and inherent site-specific exposures, naturally

occurring or production system specific chemicals capable of presenting primarily toxicity (adverse effects) are also presented as a third group and would form part of the assessment process. An additional reason for presenting this for animal health is that the first group of chemicals identified also provides scientific data used to formulate human toxicological standards [16; 17].

2.8.1.1 EDCs Relevant to Animal Health – Inorganic Water Quality Constituents

As noted by the World Health Organisation [18] assessment of mineral exposure and effects in animals serves a dual purpose of not only addressing animal hazard and risk assessment processes, but as many derived values for human health are based on animal studies, insight is also gained towards current guideline derivations and this process illustrates the recognized link between animal and human perspectives. The U.S. Department of Health and Humans Sciences (Food and Drug Administration) tasked the National Academy of Sciences to convene scientific experts specializing in nutrition, toxicology and veterinary sciences and provide recommendations on animal tolerances and toxic levels with considerations for production animals, companion animals, and humans [16]. Increasingly the quality of animal products destined for human consumption is also considered and the committee was also tasked with identifying elements posing potential human health concerns, with the key elements listed in Table 12.

It is also beyond the scope of this report to present published data on the exposure concentrations reported from different routes in the South African context, but the reader is referred to comprehensive exposure data via subterranean drinking water and naturally occurring earth dams observed in South African animal production systems as provided in WRC final reports listed in the references.

Table 12. Summary maximum tolerable levels of inorganic constituents routinely present in animal exposures [16, 17].

Inorganic Water Quality Constituent	Level of Concern for Animal Health	Trend in Maximum Tolerable Levels: 2005 relative to 1980
Aluminium	Low	Increased^
Antimony	Low	*New*^
Arsenic	Medium	**Decreased^**
Barium	Low	Increased^
Bismuth	Low	Increased^
Boron	Medium	Similar^
Bromine	Medium	Similar^ **Decreased***
Cadmium	High	Increased^
Calcium	Medium	**Decreased (ruminants)^** Increased (poultry)^
Chromium	Low	**Decreased^**
Cobalt	Low	Increased^
Copper	High	**Decreased^**
Fluorine	High	Increased^ **Decreased***
Germanium	Low	*New*^
Iodine	Low	Similar^
Iron	Medium	**Decreased^**
Lead	High	**Decreased (nonruminants)^** Increased (ruminants)^
Lithium	Low	*New*^
Magnesium	Low	Increased^
Manganese	Low	Increased^
Mercury	High	**Decreased^**
Molybdenum	High	Increased (swine)^
Nitrate	High	**Decreased***
Nickel	Low	Increased^
Phosphorus	Medium	**Decreased^**
Potassium	Medium	**Decreased^**
Rare earths	Low	*New*^
Rubidium	Low	*New*^
Selenium	High	Increased^ **Decreased***
Silicon	Low	Similar^
Silver	Low	Similar^
Sodium Chloride	High	**Decreased^**
Strontium	Low	**Decreased^**
Sulphur	High	Similar^
Tin	Low	*New*^
Titanium	Low	*New*^
Tungsten	Low	Similar^
Uranium	Low	*New*^
Vanadium	High	Similar^
Zinc	Medium	**Decreased^**

^ = NAS (2005) [22]
* = Trend in local and international water quality guidelines

2.8.1.2 EDCs Relevant to Animal Health – Pesticides, Herbicides, Disinfectants, Insecticides and Fungicides.

When reviewing this category collection of EDCs it is important to take note of plans to phase out many in the near future allowing for more an appropriate priority list to be formulated on which research needs and requirements may be based. International legislative changes regarding pesticide approval that may result in the withdrawal of products with possible endocrine disruption do not require only endocrine disruption evidence for motivation for removal. This is due to the active ingredients often reported to also pose carcinogenic, mutagenic or reprotoxic concerns. In some instances undesirable characteristics relating to environmental aspects may be the motivation. The classifications under which these criteria are defined relevant are:

- Category 1 or 2 mutagens;
- Category 1 or 2 carcinogens or reproductive toxins unless exposure is “negligible”;
- Endocrine disruptors which may cause adverse effects unless exposure is “negligible”;
- Persistent Organic Polluters (PoPs);
- Persistent – Bio accumulative – Toxic (PBT);
- Very persistent – very Bio accumulative (vPvB).

Numerous derogations apply, mostly where plant health and hence agricultural food security is at risk and although subject to renewable authorization the intention is to make allowances under exceptional circumstances only. Also relevant to South African agriculture is provision for approval subject to being classified as candidates for substitution, implying that approval can be withdrawn should safer alternatives be not only available but financially viable in the production context. This context is viewed differently in developing countries.

A total estimate of between 57 and 74 substances assessed are considered to be potentially lost in terms new EU legislation with the biggest variable due to uncertainty concerning criteria for endocrine disruption. By category the substances most likely to be lost, and that may be lost, are fungicides, whereas substances likely to be lost due to classification as potential candidates for substitution are herbicides and insecticides.

Table 13 presents a list of substances that are likely to be removed from use in agriculture due to considerations of the interim adopted definition of the EU for endocrine disruptors.

Table 14 presents those that may be eliminated with consideration for adverse effects such as carcinogenicity and reproductive toxicity, regardless of evidence of endocrine disruption [19] based on assessments by the German Federal Ministry of Food, Agriculture and Consumer Protection (FMFACCP), the Swedish Chemicals Agency (KEMI) and the UK Pesticides Safety Directorate (PSD) – now the Chemicals Regulation Directorate (CRD). It should be noted that many more substances that are likely to be classified as “for substitution” under the EU Directive 91/414, but as these lists are quite expansive they are not included here [20].

Table 13. List of substances most likely to be eliminated following the replacement of EU Directive 91/414 [19].

Agricultural Chemical	Hazard Criteria relevant[a]	Approval Expiry[b]
Insectides		
Bifenthrin	PBT/vPvB + Endocrine?	2018 N
Esfenvalerate	PBT	2011 N
Flufenoxuron	C2/PBT	2020 N
Lufenuron	PBT/vPvB	2018 N
Thiacloprid	Endocrine?	2014 Y
Fungicides		
Bitertanol	R2 + Endocrine	2020 Y
Carbendazim	M2/R2 + Endocrine?	2009 N
Cyprocinazole	Endocrine?	2020 Y
Dinocap	R2	2009 Y
Epoxiconazole	Endocrine?	2018 Y
Fenbuconazole	Endocrine?	2020 Y
Flusilazole	R2 + Endocrine?	N/A Y
Iprodione	Endocrine?	2012 Y
Mancozeb	Endocrine?	2016 Y
Maneb	Endocrine?	2016 Y
Metconazole	Endocrine?	2017 Y
Quinoxyfen	?	2014 N
Tebuconazole	Endocrine?	2018 Y
Herbicides		
Amitrole	Endocrine?	2011 Y
Flumioxazine	R2	2012 Y
Glufosinate	R2	2017 Y
Ioxynil	Endocrine?	2015 Y
Linuron	R2 + Endocrine?	2013 Y
Molinate	Endocrine?	2014 Y
Pendimethalin	PBT	2013 N
Tralkoxydim	Endocrine?	2019 Y

[a]? best available assessment at the present time but subject to change following crop-by-crop and pest-by-pest evaluations.
[b]Based on Annexure 1 Expiry and Renewal Timelines where N = derogation not available, Y = Derogation available

Table 14. List of substances that may be eliminated following the replacement of EU Directive 91/414 with consideration for potential endocrine disruption, carcinogenicity and reproductive toxicity [19].

Agricultural Chemical	Hazard Criteria relevant[a]	Approval Expiry[b]
Insectides		
Deltamethrin	Endocrine?	2013 Y
Dimethoate	Endocrine?	2016 Y
Fungicides		
Difenoconazole	Endocrine?	2018 Y
Folpet	Endocrine?	2017 Y
Fluquinoconazole	Endocrine?	2020 Y
Fuberidazole	Endocrine?	2019 Y
Metiram	Endocrine?	2015 Y
Mycolbutanil	Endocrine?	2020 Y
Penconazole	Endocrine?	2019 Y
Prochloraz	Endocrine?	2020 Y
Propiconazole	Endocrine?	2013 Y
Prothioconazole	Endocrine?	2018 Y
Tetraconazole	Endocrine?	2019 Y
Tiram	Endocrine?	2013 Y
Triadimenol	Endocrine?	2018 Y
Triticonazole	Endocrine?	2017 Y
Herbicides		
2,4 D	Endocrine?	2011 Y
Carbetamide	Endocrine?	2020 Y
Chlortoluron	Endocrine?	2016 Y
Flumetron	Endocrine?	2015 Y
Metribuzin	Endocrine?	2013 Y
Picloram	Endocrine?	2014 Y
Tepraloxydim	Endocrine?	2016 Y
Trisulfuron	Endocrine?	2019 Y
Soil Sterilant		
Metam	Endocrine?	2019 Y

[a]? best available assessment at the present time but subject to change following crop-by-crop and pest-by-pest evaluations.
[b]Based on Annexure 1 Expiry and Renewal Timelines where N = derogation not available, Y = Derogation available

Adapted results of a recent review of chemicals classified as High Concern based on health endpoints is presented in Table 15 [21]. The table presents those chemicals with endocrine disruption and development as a health endpoint (development may be argued to also involve endocrine system in later stages).

Table 16 provides an indication of other endpoints and chemical source to indicate the relative chemical to health endpoint types. As is discussed in the assessment section later risk assessment considerations require a source exposure perspective, and some chemicals with endocrine disruption endpoints are actually considered due to impurities in the chemical and not necessarily the active ingredient.

Table 15 List of Agriculture Use substances classified as Chemicals of High Concern with Endocrine and Developmental endpoints (adapted from [19]).

Chemicals[a]	Health Endpoint	Types of Use
1,1,1,2-Tetrachloro-2,2-bis(4-chlorophenyl)ethane [3563-45-9] 1,1,1-Trichloro-2,2-bis(chlorophyenyl)ethane [2971-22-4] 2,3-dichloroaniline [95-76-1] 3-OH-o,p'-DDT [43216-70-2] 4-Cyclohexylphenol [1131-60-8] 4-Nitrotoluene [99-99-0] Beta-HCH [319-85-7] Bifenthrin [82657-04-3] Boric acid [10043-35-3] Carbaryl [63-25-2] Cis-Nonachlor [5103-73-1] Cyclotetrasiloxane, octamethyl- [556-67-2] Dicofol [115-32-2] Endosulfan [115-29-7] Fenarimol [60168-88-9] Fenitrothion [122-14-5] Fentin acetate [900-95-8] Ioxynil [1689-83-4] Methoxychlor [72-43-5] n-Butyl p-Hydroxybenzoate [94-26-8] Nonylphenolethoxylate [9016-45-9] n-propyl p-hydroxybenzoate [94-13-3] o,p'-DDMU [14835-94-0] Omethoate [1113-02-6] Octyphenol [27193-28-8] Phenol, nonyl [25154-52-3] p-Hydroxybenzoic acid [99-96-7] Quinalphos [13593-03-8] Stannane [4342-36-3] Stannane, tributyl [688-73-3] Terbutryn [886-50-0] Trans-Nonachlor [39765-80-5] Trifluralin [1582-09-8] Zineb [12122-67-7]	Endocrine System	Pesticide Impurity; Chemical intermediate for herbicide; Herbicide; Pesticide; Pesticide breakdown product; Antimicrobial additive; Agricultural chemicals; Pesticide breakdown product; Bacteriacide
2,4-D butyric acid [94-82-6]	Endocrine system, Reproduction	Pesticide
Furan & Dioxin Amitrole [61-82-5] Benzene,1,1'-(2,2-dichloroethylidene)bis[4-chloro- [72-54-8] Chlordane [57-74-9] Chlordimeform [6164-98-3] Hexachlorocyclohexane, gamma- [58-89-9] Linuron [330-55-2] Mancozeb [8018-01-7] Maneb [12427-38-2] Mirex [2385-85-5] Nitrofen [1836-75-5] Toxaphene [8001-35-2]	Endocrine System Cancer	Contaminant of some pesticides during manufacturing process. Pesticide

Chemicals[a]	Health Endpoint	Types of Use
Benzene,1-chloro-2-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl]- [789-02-6]	Endocrine system, Reproduction, Development.	Pesticide
Chlordecone [143-5-0] Hexachlorobenzene [118-74-1] Metham sodium [137-42-8] Metiram [9006-42-2] Resmethrin [10453-86-8]	Endocrine system Cancer, Development.	Pesticide
Epichlorohydrin [106-89-8]	Endocrine system, Cancer, Reproduction	Fumigant
Warfarin [81-81-2]	Development, Endocrine system	Pesticide
Hydrogen cyanide [74-90-8]	Endocrine system, Nervous system	Pesticide
Dicamba [1918-00-9] Dichlorophene [97-23-4] Dicrotophos [141-66-2] Dicumarol [66-76-2] Dinocap [39300-45-3] Diquat dibromide [85-00-7] Endrin [72-20-8] Fenoxaprop ethyl [66441-23-4] Fluazifop butyl [69806-50-4] Flutolanil [66332-96-5] Fluvalinate [69409-94-5] Glyphospate [1071-83-6] Methazole [20354-26-1] Metalochlor [51218-45-2] Nabam [142-59-6] Potassium-dimethyldithiocarbamate [128-03-0] Terbacil [5902-51-2] Triforine [26644-46-2]	Development	Pesticide, Insecticide
Diclofop methyl [51338-27-3] Heptachlor [76-44-8] Nitrapyrin [1929-82-4] Oxadiazon [19666-30-9] Oxythioquinox [2439-01-2] Stannane, hydroxytriphenyl [76-87-9]	Cancer, Development	Pesticide Fertilizer
1, 2-Dibromo-3-chloropropane [96-12-8] 1, 3-Dichloropropene [542-75-6] Atrazine [1912-24-9] Benzene,1,1'(dichloroethylidene)bis[4-chloro- [72-55-9]	Cancer, Endocrine system, Respiratory system, Reproduction, Cardiovascular system	Pesticide
2,4,5-Trichlorophenoxyacetic acid [93-76-5] Nitrite [14797-65-0]	Blood, Development	Pesticide Fertilizer waste
Acetochlor [34256-82-1] Alachlor [15972-60-8] Cyanazine [21725-46-2] Dieldrin [6057-1] Dyphonate [944-22-9] Ethylene dibromide [106-93-4] Ethylene oxide [75-21-8] N-methylformamide [123-39-7] Pentachlorophenol [87-86-5] Pichloram [1918-02-1] Tetramethyl thiuram disulphide [137-26-8]	Cancer, Development, Endocrine system, Kidney, Liver, Nervous system, Respiratory system, Reproduction	Pesticide Pesticide manufacturing Growth regulator
Amitraz [33089-61-1] Avermectic B1 [65195-55-3] Benomyl [17804-353-2] Chlorsulfuron [64902-72-3] Cycloate [1134-23-2] Cycloheximide [66-81-9] Dinoseb [88-85-7] Hydramethylonon [67485-29-4] Myclobutanil [88671-89-0] Quizalofop-ethyl [76578-14-8] Streptomycin sulphate [3810-74-0] Streptozocin [18883-66-4] Triadimefon [43121-43-3]	Development, Reproduction	Pesticide, veterinary pharmaceutical

Chemicals[a]	Health Endpoint	Types of Use
Calcium cyanide [592-01-8] Carbon disulphide [75-13-0] Carbon disulphide [75-13-0] Chlorthal-dimethy [1861-32-1] Fluridone [59756-60-4]	Nervous system, Thyroid, Weight loss, Development, Kidney	Pesticide
Dicyclopentadiene [77-73-6] Hexythiazox [78587-05-0]	Adrenal glands, Eyes, Kidney, Liver, Skin	Pesticide
Dodine [2439-10-3] Norflurazon [27314-13-2]	Thyroid, Liver	Pesticide
Methyl bromide [74-83-9]	Development, Nervous system, Respiratory, Gastrointestinal system	Fungicide

[a] [ ] = CAS Number

Table 16 Summary of endpoint types and source of Chemicals classified as High Concern (adapted from [19]).

Summary of Endocrine System endpoint chemicals		
Agriculture-related Chemicals n = 65	**Non-Agriculture related Chemicals** n = 75	**Examples of Non-Agriculture related chemicals:** Acid base indicators; Accelerator (rubber production); Anti-oxidants; Bleaching agents; Combustion by-products; Corrosion inhibitors; Cosmetics; Disinfection by-products; Detergents (non-ionic); Dye carriers; Dye intermediates; Flame retardents; Food flavouring; Hair care; Heat stabilizer; Heat transfer medium; Industrial manufacturing (resins, plastics, bonding agents); Plasticizers; Pharmaceuticals; Preservatives, Photography; PCBs; Petroleum-related products and by-products; Reactants; Reducing agents; Rust inhibiting agents; Surfactants; Soldering flux; Stabilizers; UV Protectants; Vulcanisation of rubbers; Water purification; Weather proofing.
Endpoints = Endocrine only **n = 29**	**Endpoints =** Endocrine & other **n = 38**	**Endpoints =** Blood, Body weight, Bone marrow, Cardiovascular system, Cancer, Development, Eyes, Gastro-intestinal system, Immune system, Liver, Nervous system, Organs, Reproduction, Respiratory system, Spleen, Environmentally persistent/bioaccumulative/toxic **n = 260**

2.8.1.3 EDCs Relevant to Animal Health – Naturally Occurring or Production System Specific

Consideration for background exposure to other toxins is required when assessing the hazards and risks from exposure to an EDC. The primary reasons for this are that some classes of toxins may indirectly compromise the homeostatic mechanisms required for normal endocrine function (e.g. compromised renal function) and in many cases the clinical signs of primary toxicity may mask underlying endocrine disruption. In some cases both primary toxicity and endocrine disruption are recognized effects. It is also necessary to differential between naturally occurring EDCs that may be present (e.g. in the feed and water) and those introduced by the production system (e.g. disinfectants and supplements).

Although the complexities of sources, toxicokinetics, mode of action, risk factors, clinical signs, lesions, diagnostic testing and prevention and control, are too complex to be presented here the reader should take note of the baseline exposures and refer to veterinary toxicology literature for further information when investigating EDC exposures. This is fundamental to unraveling cause and effect relationships and performing a differential diagnosis.

To illustrate the contents relevant Table 17 provides only a selected few of the exposure considerations with the full list too extensive for presentation here.

Table 17. Considerations for exposure when assessing effects of EDCs on animal health (adapted from [22; 17; 23]).

Category	Compound	Exposure	Effects
Feed-associated toxicants	**Nitrate**	Nitrate/nitrite poisoning exposure is documented through the increased concentrations in feed, forage and water associated with fertilizer applications and contamination linked to confined animal feeding operations. Numerous species-specific and production system risk factors are relevant	Primary historic focus has been on methemoglobin production-related fatalities and spontaneous abortions, but increased evidence is reported for endocrine disrupting effects
	Others: Ammoniated feed; Gossypol; Ionophores; Non-protein nitrogen; and Sulphur.		
Industrial and process products	**Boric acid**	Used in pharmaceutical preparations and as antibacterial and antifungal agents. Ingestion of powders used in bait traps has been reported	Primarily cytotoxic but testicular degeneration and teratogenic effects noted. Clinical signs may include diarrhea and abdominal pain and signs associated with renal tubular necrosis. Clinical pathology indicative of microcytic hypochromic anemia, hypernatremia, hyperchloremia, metabolic acidosis and hyperkalemia
	Others: Bleaches; Detergents; Diphyridyl Herbicides: Fertilizers; Glycophosphate Herbicides; Naphthalene; Phenols; and Propylene Glycol		
Insecticides and Molluscicides	**Amitraz**	A synthetic acaricide for topical control of ticks, mites and lice on many animals. Often applied as a pour-on, dip, collar or shampoo	Sedative effects due to alpha-2 adrenergic agonist activity in the central nervous system. Mydriasis and bradycardia reported. Equines regarded as sensitive
	Others: Anticholinesterase insecticides; Diethyltoluamide; and Metaldehyde.		
Novel insecticides	**Organo-chlorine insecticides**	Chlorinated hydrocarbons of relevance to animals include numerous insecticides, but primary ones include diphenyl aliphatic compounds (DDT, methoxychlor, perthane and dicofol), aryl hydrocarbons (lindane, mirex, kepone, and paradichlorobenzene) and cyclodiene insecticides (aldrin, dieldrin, endrin, chlordane, heptachlor and toxaphene), many of which are either banned or highly restricted. Used for pest control.	Lipid soluble compounds which are rapidly absorbed with acute toxicosis and bioaccumulation relevant. Many decrease resting membrane potential resulting in neuron stimulation. Signs predominantly associated with the nervous system, but also endocrine disruption
	Others: Fipronil; Imidacloprid; Hydramethylnon; Sulfluramid; Organochlorine insecticides; and Pyrethrins and Pyrethroids.		
Metals and Minerals	The list of inorganic elements relevant to animal health is growing with increasing recognition of low-dose chronic effects and adverse effects other than classic reported toxicities (endocrine disruption and induced deficiencies) with the main elements presented in Table 1.13. The complete list is too extensive to review here and the reader is referred to both toxicology and animal nutrition reviews. Of primary relevance to EDCs however is the recognition of potentially hazardous inorganic constituents in drinking water, soil and feed exposure routes noted in South African production systems leading to the adequate description of background exposure values essential in any site-specific study investigating agricultural chemicals and health (WRC Final Reports, 301; 644; 875 & 1175). Application to relevant elements via injectable preparations is also growing as is the addition of supplements containing these elements to the drinking water.		
Other Categories: **Pharmaceuticals (Antimicrobials; Antineoplastics; Antiparasiticals).** Although mostly of indirect concerns it should be noted that the following preparations may be widely used in animal production: Analgesics; Anticonvulsants; Antidepressants; Antihistamines; Bronchodilators; Cardiovascular drugs; Decongestants; Diabetic medications; Hypothyroid medications; Methylxanthines and Muscle relaxants. **Rodenticides and Avicides (3-Chloro-p-Toluidine Hydrochloride; 4-Aminopyridine;Anticoagulant Rodenticides; Bromethalin; Cholecalciferol; Fluoroacetate; Strychnine; Zinc Phosphide).** **Other classes:** Not directly linked to agricultural chemicals, but relevant to production systems, including mycotoxins and plant compounds, such as oxalates, tannic acid, alkaloids, glycosides, phytoestrogens and steroidal saponins.			

2.8.1.4 Examples of General EDC Lists

The preceding section provides animal health as a reference point for illustrating the complexities regarding types of EDCs. It should be appreciated that investigating the exposure to EDCs for humans is arguably more complex as in many cases animal exposures are easier to quantify as they occur in confined areas with limited source variability. This is characteristic of intensive commercial production systems where formulated rations, veterinary product usage and water supplies are strictly managed, and a central motivating factor for the use of commercially produced livestock as sentinels.

As an indication of some of the wide range of potential types of EDCs relevant to human exposure Table 18 presents various categories typically found in the international government EDC programs. The First List of the US EPA Endocrine Disruptor Screening Progam is presented in Table 19 and the Second List in Table 20 as the most recent lists published and specifically states that the chemicals listed were "…selected on the basis of exposure potential only…" and as such "..it should not be construed or characterized as a list of known or likely endocrine disruptors" [24].

Reasons for the different lists are too numerous to be presented here and are contained in the US EPA reference website [24], but it is noteworthy that the First List appeared 11 years after the program started and the Second List in under 6 months of the publication of the First List. The US EPA states that it suggests the Second List should at least be included for screening purposes. Although not presented in depth in this Volume it is noteworthy that a comparison between international EDCs varies significantly. As evidenced in Tables 19 and 20 the US EPA does not include many reported EDCs in the First List (e.g. Lindane or Vinclozolin).

An additional reason for the lists varying with respect to agricultural chemicals, notably pesticides, herbicides and insecticides, is that if a compound is banned in a specific country then after a phase-out period the compound is not necessarily relevant in environmental exposure media. A more recent review from the EU indicates that numerous pesticides are to be banned, and although they may not be necessarily banned in South Africa, due to the reliance on trade in agricultural products with the EU, continued use of the product may decline significantly. This could warrant either the exclusion thereof from a monitoring list or the lowering of the priority assigned to the compound.

In order to present a South African perspective a comparative list to Tables 18, 19 and 20 is provided in Table 21 as an indication of some specific chemicals for which routine water analysis can be performed. It is necessary to differentiate between routine analytical procedures and project-specific or targeted analysis which may be performed on a request basis, usually at a high cost. A list of relevant laboratories and EDC-relating testing is provided in Table 22. Problems occur in the limits of detection, extraction procedures and the ability to routinely handle large numbers of samples. As noted in a report to the WRC concerning EDC monitoring many laboratories are available that can perform some chemical analysis for selected EDCs but some capacity problems are often encountered and some laboratories are no longer able to perform the desired tests [25]. In some

cases analysis of total content as opposed to selected species is performed, for example, DDT. The reader is referred to the list of WRC Projects listed in the Recommended Reading section at the end of this volume for the relevant reports, but it is noteworthy that currently little or no monitoring data exists for many EDCs (notably pesticides) in South African water systems [25].

The EDC screening techniques currently available are presented in Volume 3 of this Manual and the reader is referred to that volume for further detail. It is notable that in South Africa only Phenols are listed as a standard for drinking water quality [26] and only Atrazine and Phenol are listed in the 1996 South African Water Quality Guidelines for Domestic Use [2] with Target Water Quality Ranges. Collectively, the US EPA, WHO and Australia list a total of 77 different pesticides as having some form of drinking water guideline or standard [4, 3, 5]. Much criticism has been published regarding the lack of public health attention that pesticides in water sources and drinking water receive in South Africa with the primary reasons often cited as high costs, technical constraints and a shortage of appropriately skilled laboratory staff. These consequently led to the lack of monitoring data on which to base regulatory policies and frameworks [27].

By comparison 14 pesticides are listed for Aquatic Ecosystem health in South Africa, however, Canada cites 34 for the same water use [6]. Guidelines or standards differ markedly between countries and are largely a function of different capacities regarding limits of detection.

Table 18. Examples of EDC exposure when assessing effects of EDCs on human health.

Category	
Persistent organohalogens	
Compound	**Relevant Endocrine System**
Benzenehexachloride (BHC); Octachlorostyrene; PBBs; PCB, hydroxylated; PBDEs; Pentachlorophenol#	Thyroid
1,2-dibromoethane; Chloroform	Reproductive
Dioxins and Furans*#: in order of anti-oestrogenic potency: 2,3,7,8-tetrachlorodibenzo-p-dioxin; 2,3,7,8-tetrachlorodibenzofuran; 2,3,4,7,8-pentachlorodibenzo-furan; 1,2,3,7,9-pentachlorodibenzofuran; 1,3,6,8-tetrachlo-rodibenzofuran. PBBs.	Oestrogen
PCBs: in order of antiestrogenic potency: 3,3' –pentachlorobiphenyl; 3,3,4,4,5,5'-hexachlorobiphenyl; 3,3',4,4-tetrachlorobiphenyl 2,3,3',4,4',5'-hexa, 2,3,3',4,4'- and 2,3,4,4',5-pentachlorobiphenyl.	Oestrogen/ Androgen /Thyroid. Adverse reproductive effects
Food Antioxidant	
Butylated hydroxyanisole#	Oestrogen
Pesticide	
Acetochlor; Alachlor; Amitrol#; Chlofentezine; Fenbuconazole; Fipronil; Heptachlor*#; Heptachlor-epoxide; Karate; Malathion#; Mancozeb; Maneb; Methomyl; Metribuzen; Nitrofen#; Pendimethalin; Pentachloronitrobenzene#; Prodiamine; Pyrimethanil; Tarstar; Thiazopyr; Thiram#; Toxaphene*#; Zineb#; Ziram#	Thyroid
Aldrin; Allethrin, d-trans; DDT^#; Dicofol; Dieldrin*#; Endosulfan#; Ethylene thiourea#; Fenarimol; Fenvalerate; Kepone#; Lindane#; Methoxychlor#; Nonachlor-trans; Permethrin; Toxaphene*#; Triadimefon; Triadimenol	Oestrogen
Atrazine#; Thiram#	Neuroendocrine-pituitary (depression of LH surge), testosterone metabolism
Carbaryl	Oestrogen and progesterone
Chlordane	Testosterone and progesterone
Cypermethrin; Ketoconazole; Oxychlordane; Tributyltin	Reproductive
DDT metabolite p,p'-- DDE#; Lindane#; Linuron; Procymidone; Sumithrin; Vinclozolin	Androgen
Fenitrothion; Mirex*#	Antiandrogen
Heptachlor-epoxide	Thyroid /Reproductive
Iprodione	Testosterone synthesis inhibition
Phthalate	
Butyl benzyl phthalate#; Diethyl phthalate#	Oestrogen
Di-n-butyl phthalate#; Di-ethylhexyl phthalate#	Oestrogen / Androgen
Other	
Benzophenone#; Bisphenol A#; Bisphenol F; Nonylphenol / octylphenol; Styrene dimers and trimers	Oestrogen
Benzo(a)pyrene#	Androgen
Carbendazim Ethane Dimethane Sulphonate Perflurooctane sulfonate	Reproductive
Resorcinol	Thyroid
Metals	
Arsenic (arsenite#)	Glucocorticoid
Cadmium#	Oestrogenic
Lead	Reproductive
Mercury	Thyroid / Reproductive
^ = usage restricted by Stockholm Convention on Persistent Organic Pollutants – call to phase out * = usage banned by Stockholm Convention on Persistent Organic Pollutants # = frequently listed as a common EDC	

Table 19. First List of Tier 1 List of chemicals to be screened for the US EPA Endocrine Disruptor Screening Program [24].

Chemical Name	**CAS Number**	**Pesticide Active ingredient (PA) or HPV/Inert (HPVI)**
2,4 D	94757	**PA**
4,7-Methano-1H-isoindole-1,3(2H)-dione,2-(2-ethylhexyl)-3a,4,7,7a-tetrahydro-	113484	PA
Abamectin; Acephate; Atrazine	71751412; 30560191; 1912249	PA
Acetone	67641	HPVI
Benfluralin; Bifenthrin	1861401; 82657043	PA
Butyl benzyl phthalate	85687	HPVI
Captan; Carbamothioic acid, dipropyl-, S-ethyl ester; Carbaryl; Carbofuran	133062; 759944; 63252; 1563662	PA
Chlorothalonil; Chlorpyrifos; Cyfluthrin; Cypermethrin	1897456; 2921882; 68359375; 52315078	PA
DCPA (or chlorthal-dimethyl); Diazinon	1861321; 333415	PA
Dibutyl phthalate	84742	HPVI
Dichlobenil; Dicofol; Disulfoton	1194656; 115322; 298044	PA
Diethyl phthalate	84662	HPVI
Dimethoate	60515	PA
Dimethyl phthalate; Di-sec-octyl phthalate	131113; 117817	HPVI
Endosulfan; Esfenvalerate; Ethoprop	115297; 66230044; 13194484	PA
Fenbutatin oxide; Flutolanil; Folpet	13356086; 66332965; 133073	PA
Gardona (cis-isomer); Glyphosate	22248799; 1071836	PA
Imidacloprid; Iprodione	138261413; 36734197	PA
Isophorone	78591	HPVI
Linuron	330552	PA
Malathion; Metalaxyl; Methamidophos; Methomyl; Methyl parathion; Metolachlor Metribuzin; Myclobutanil	121755; 57837191; 10265926; 16752775; 298000; 51218452; 21087649; 88671890	PA
Methyl ethyl ketone	78933	HPVI
Norflurazon	27314132	PA
o-Phenylphenol; Oxamyl	90437; 23135220	PA
Permethrin; Phosmet; Piperonylbutoxide; Propachlor; Propargite; Propiconazole; Propyzamide; Pyridine, 2-(1-methyl-2-(4-phenoxyphenoxy)ethoxy)-	52645531; 732116; 51036 1918167; 2312358; 60207901; 23950585; 95737681	PA
Quintozene	82688	PA
Resmethrin	10453868	PA
Simazine	122349	PA
Tebuconazole	107534963	PA
Toluene	108883	HPVI
Triadimefon; Trifluralin	43121433; 1582098	PA

Table 20. First List of Tier 1 List of chemicals to be screened for the US EPA Endocrine Disruptor Screening Program [24].

Chemical Name	CAS Number
1,1,1,2-Tetrachloroethane; 1,1,1-Trichloroethane; 1,1,2-Trichloroethane	630-20-6; 71-55-6; 79-00-5
1,1-Dichloroethane; 1,1-Dichloroethylene	75-34-3; 75-35-4
1,2,3-Trichloropropane; 1,2,4-Trichlorobenzene	96-18-4; 120-82-1
1,2-Dibromo-3-chloropropane (DBCP); 1,2-Dichloroethane; 1,2-Dichloropropane	96-12-8; 107-06-2; 78-87-5
1,3-Dinitrobenzene	99-65-0
1,4-Dioxane	123-91-1
1-Butanol; 2,4,5-TP (Silvex); 2-Methoxyethanol; 2-Propen-1-ol	71-36-3; 93-72-1; 109-86-4; 107-18-6
4,4'-Methylenedianiline	101-77-9
Acetaldehyde; Acetamide; Acetochlor; Acetochlorethanesulfonic acid (ESA); Acetochloroxanilic acid (OA)	75-07-0; 60-35-5; 34256-82-1; 187022-11-3; 194992-44-4
Acrolein; Acrylamide; Alachlor; Alachlorethanesulfonic acid (ESA); Alachloroxanilic acid (OA); alpha-Hexachlorocyclohexane	107-02-8; 79-06-1; 15972-60-8; 142363-53-9; 171262-17-2; 319-84-6
Aniline	62-53-3
Bensulide; Benzene; Benzo(a)pyrene (PAHs); Benzyl chloride; Butylatedhydroxyanisole	741-58-2; 71-43-2; 50-32-8; 100-44-7; 25013-16-5
Carbon tetrachloride; Chlordane; Chlorobenzene; cis-1,2-Dichloroethylene; Clethodim; Clofentezine; Clomazone.	56-23-5; 57-74-9; 108-90-7; 156-59-2 99129-21-2; 74115-24-5; 81777-89-1
Coumaphos; Cumenehydroperoxide; Cyanamide; Cyromazine	56-72-4; 80-15-9; 420-04-2; 66215-27-8
Dalapon; Denatonium saccharide; Di(2-ethylhexyl) adipate; Dichloromethane; Dicrotophos; Dimethipin; Dinoseb; Diuron	75-99-0; 90823-38-4; 103-23-1; 75-09-2; 141-66-2; 55290-64-7; 88-85-7; 330-54-1
Endothall; Endrin; Epichlorohydrin; Erythromycin	145-73-3; 72-20-8; 106-89-8; 114-07-8
Ethylbenzene; Ethylene dibromide; Ethylene glycol; Ethylene thiourea; Ethylurethane; Etofenprox	100-41-4; 106-93-4; 107-21-1; 96-45-7; 51-79-6; 80844-07-1
Fenamiphos; Fenarimol; Fenoxaprop-P-ethyl; Fenoxycarb; Flumetsulam; Fomesafen sodium; Fosetyl-Al (Aliette)	22224-92-6; 60168-88-9; 71283-80-2; 72490-01-8; 98967-40-9; 108731-70-0; 39148-24-8
Glufosinate ammonium	77182-82-2
HCFC-22; Heptachlor; Heptachlor epoxide; Hexachlorobenzene; Hexachlorocyclopentadiene; Hexane; Hexythiazox; Hydrazine	75-45-6; 76-44-8; 1024-57-3; 118-74-1; 77-47-4; 110-54-3; 78587-05-0; 302-01-2
Isoxaben	82558-50-7
Lactofen; Lindane	77501-63-4; 58-89-9
Methanol; Methoxychlor; Methyl tert-butyl ether; Metolachlorethanesulfonic acid (ESA); Metolachloroxanilic acid (OA); Molinate	67-56-1; 72-43-5; 1634-04-4; 171118-09-5; 152019-73-3; 2212-67-1
Nitrobenzene; Nitroglycerin; N-Methyl-2-pyrrolidone; -Nitrosodimethylamine (NDMA); n-Propylbenzene	98-95-3; 55-63-0; 872-50-4; 62-75-9; 103-65-1
o-Dichlorobenzene; o-Toluidine; Oxirane, methyl; Oxydemeton-methyl; Oxyfluorfen	95-50-1; 95-53-4; 75-56-9; 301-12-2; 42874-03-3
Paclobutrazol; p-Dichlorobenzene; Pentachlorophenol; Perchlorate; Perfluorooctane sulfonic acid (PFOS); Perfluorooctanoic acid (PFOA); Picloram	76738-62-0; 106-46-7; 87-86-5; 14797-73-0; 1763-23-1; 335-67-1; 1918-02-1
Polychlorinated biphenyls; Profenofos; Propetamphos; Propionic acid; Pyridate.	1336-36-3; 41198-08-7; 31218-83-4; 79-09-4; 55512-33-9
Quinclorac; Quinoline; Quizalofop-P-ethyl	84087-01-4; 91-22-5; 100646-51-3
RDX	121-82-4
sec-Butylbenzene; Sodium tetrathiocarbonate; Styrene; Sulfosate	135-98-8; 7345-69-9; 100-42-5; 81591-81-3
Temephos; Terbufos; Terbufossulfone; Tetrachloroethylene; Thiophanate-methyl; Toluene diisocyanate; Toxaphene; trans-1,2-Dichloroethylene; Trichloroethylene; Triethylamine; Triflumizole; Trinexapac-ethyl; Triphenyltin hydroxide (TPTH)	3383-96-8; 13071-79-9; 56070-16-7; 127-18-4; 23564-05-8; 26471-62-5; 8001-35-2; 156-60-5; 79-01-6; 121-44-8; 68694-11-1; 95266-40-3; 76-87-9
Vinclozolin	50471-44-8
Xylenes (total)	1330-20-7
Ziram	137-30-4

Table 21. Examples of routine water testing for EDCs in South Africa.

Endocrine Disrupting Chemical	Laboratory Routine Test
Aldrin	SABS: Food and Water Chemistry Rand Water
Dieldrin	SABS: Food and Water Chemistry Rand Water
Endrin	SABS: Food and Water Chemistry Rand Water
2,4-D (2,4-Dichlorophenoxyacetic acid)	SABS: Food and Water Chemistry Rand Water
MCPA (2-methyl-4-chlorophenoxyacetic acid)	SABS: Food and Water Chemistry Rand Water
Atrazine	SABS: Food and Water Chemistry Rand Water
Simazine	SABS: Food and Water Chemistry Rand Water
Terbutylazine	SABS: Food and Water Chemistry Rand Water
Naphthalene	Rand Water
Acenaphthylene	Rand Water
Acenaphthene	Rand Water
Fluorene	Rand Water
Phenanthrene	Rand Water
Anthracene	Rand Water
Fluoranthene	Rand Water
Pyrene	Rand Water
Benz[a]anthracene	Rand Water
Chrysene	Rand Water
Benzo[b]fluoranthene	Rand Water
Benzo[k]fluoranthene	Rand Water
Benzo[a]pyrene	Rand Water
Indeno[1,2,3-cd]pyrene	Rand Water
Dibenz[a,h]anthracene	Rand Water
Benzo[g,h,i]perylene	Rand Water
a-BHC	Rand Water
HCB	Rand Water
g-BHC (Lindane)	Rand Water
b-BHC	Rand Water
d-BHC	Rand Water
Vinclozolin	Rand Water
Heptachlor	Rand Water
Epoxyheptachlor	Rand Water
trans-Chlordane	Rand Water
o,p-DDE	Rand Water
cis-Chlordane	Rand Water
Endosulphan I	Rand Water
p,p-DDE	Rand Water
o,p-DDD	Rand Water
Endosulphan II	Rand Water
p,p-DDD	Rand Water
p,p-DDT	Rand Water
Endrin Aldehyde	Rand Water
Endosulphan Sulphate	Rand Water
Endrin Ketone	Rand Water
Methoxychlor	Rand Water
Chlorpyriphos	Rand Water
PCB-153	Rand Water
Phenol	Rand Water CTSSB (Total Dissolved)
2-Chlorophenol	Rand Water
2-Nitrophenol	Rand Water
4-Chloro-3-methylphenol	Rand Water
2,4,6-Trichlorophenol	Rand Water

Table 21 (cont). Examples of routine water testing for EDCs in South Africa.

Endocrine Disrupting Chemical	Laboratory Routine Test
2,4-Dinitrophenol	Rand Water
4-Nitrophenol	Rand Water
2-Methyl-3,5-Dinitrophenol	Rand Water
Pentachlorophenol	Rand Water
Chloroform	Rand Water
Dichlorobromomethane	Rand Water
Chlorodibromomethane	Rand Water
Bromoform	Rand Water
Benzene	Rand Water
Toluene	Rand Water
Ethylbenzene	Rand Water
Xylene isomers	Rand Water
Hydrazine	CTSSB (effluent
Arsenic	SABS: Food and Water Chemistry Rand Water ISCW PAL CTSSB (effluent & hydro-environmental)
Cadmium	SABS: Food and Water Chemistry Rand Water ISCW PAL CTSSB
Lead	SABS: Food and Water Chemistry Rand Water ISCW PAL CTSSB
Nitrate	SABS: Food and Water Chemistry Rand Water ISCW PAL CTSSB
Bromide	ISCW PAL
Fluoride	SABS: Food and Water Chemistry Rand Water ISCW PAL CTSSB
Selenium	SABS: Food and Water Chemistry Rand Water ISCW PAL CTSSB (effluent & hydro-environmental)
Mercury	SABS: Food and Water Chemistry Rand Water ISCW PAL CTSSB (effluent & hydro-environmental)

CTSSB = Cape Town Scientific Services Branch
ISCW = Institute for Soil, Climate and Water (Agricultural Research Council)
SABS = South African Bureau of Standards
PAL = Pelindaba Analytical Laboratories (NECSA)

Table 22. Examples of laboratories performing EDC-related testing in South Africa (adapted from [25]).

Laboratory / Institution	EDC-Related Tests
Pelindaba Analytical Laboratories (NECSA)	Inorganic Chemistry
AMPATH	Hormones: Phenols PCBs Pesticides Herbicides PAHs Inorganic Chemistry
ARC: Plant Protection Research Institute	Pesticides Herbicides Heavy metals
ARC: Institute for Soil, Climate and Water	Inorganic chemistry
ARC: Onderstepoort Veterinary Institute	Toxicant Tests
CSIR (Biochemtek) – SABS	Industrial Chemicals: Alkylphenols Phthalates PCBs
SABS: Food & Water Chemistry	Pesticides Herbicides Inorganic Chemistry
Johannesburg Water	Toxicant Tests Phenols PCBs Pesticides Herbicides Inorganic Chemistry
Rand Water	Inorganic Chemistry Pesticides Herbicides Toxicant Tests
Umgeni Water	Inorganic Chemistry Pesticides Herbicides Toxicant Tests
Waterlab	Toxicant Tests Inorganic Chemistry
Cape Town Scientific Services Branch	Inorganic Chemistry PCBs Pesticides Herbicides
University of Pretoria CSIR (Environmentek) University of Stellenbosch North-West University DWA	EDC Activity Tests

2.9 Summary

The International Programme on Chemical Safety [9] reviewed scientific peer-reviewed literature on the associations between environmental exposures and AEs that have either been demonstrated or hypothesized via endocrine disruption. In their Global Statement they observed that until more knowledge on the molecular events concerning hormone response and the potential for adverse effects becomes available, linking AEs to endocrine-mediated pathways will remain not only difficult but also controversial.

They also observed that the weakest link in determining the relationship between EDCs and AE in both humans and wildlife is the lack of exposure data, specifically during the critical development periods. It is also stated that due to the involvement of multiple systems in endocrine disruption, and the tendency for EDCs to affect diseases of multifactorial etiology, such as infertility, cancer, and neurological deficits, attributing AE to EDCs within traditional epidemiological studies is challenging.

Several years later in 2009 the Endocrine Society of America released an official statement document on EDCs [8] in which many of the same concerns and research needs and recommendations as noted by the IPCS in 2002 were highlighted. The 2009 statement from the Endocrine Society raised several key points. Firstly, it stated that "the evidence for adverse reproductive outcomes from exposure to EDCs is strong, and there is mounting evidence for effects on other endocrine systems, including thyroid, neuroendocrine, obesity and metabolism, and insulin and glucose homeostasis".

It also stated the official stance of the society was to not only adopt the "Precautionary Principle" as a critical step to enhancing human endocrine health in the face of the many key knowledge gaps, but that the Endocrine Society would "lobby for regulation seeking to decrease human exposure to the many endocrine-disrupting agents".

Lastly, the society observed that since direct causal links between exposure to EDCs and AEs were difficult to draw "screening for exposures and targeting at risk groups is a high priority". Some of the newer aspects receiving attention include:

- transgenerational effects (heritability) due to overt mutation or subtle modifications of gene expression independent of mutation – referred to as epigenetic effects;
- mixed types of effects – having both oetrogenic and antiandrogenic effects due to an EDC being metabolized into subproducts with different properties;
- mixed levels of effects – AEs on receptors, synthesis, and metabolism;
- nonspecific interference – as systems are linked disruption of one system may impact negatively on another indirectly.

As noted in the GWRC 2012 review [10] the contribution of water must not only be investigated, but the perspective thereof to the total exposure should be placed in context, with much more research required. It was also observed that:

- A meta-analysis should still be performed for assays investigating androgenic and progestagenic activity, and these have still not focused on water sample testing.
- Steriodogenesis interference as an endpoint still requires some basic science before measurements thereof can be meaningfully applied to water testing.
- The relevance of thyroid assays to water testing is still unclear.
- Despite the recognition of glucocorticoid, retinoid X receptor and peroxisome proliferator receptor as priority endocrine endpoints, little is known about their activity in water.
- Most non-oestrogenic assays have not been applied in a comprehensive manner to wastewater, with a lack of information on their activity in other waters, including drinking, surface and groundwater.

The Endocrine Society continued to present recommendations not just for further research but also for Clinical Practice. This is seen as a significant step in public communication and involvement of clinicians to gather valuable epidemiological data.

The conclusion is that the trend over years in published reviews on EDCs suggests that concerns regarding EDCs, human and wildlife health, and the environment, are warranted. This recognition extends toward practical application in a clinical environment linked ultimately to concerted efforts to reduce environmental exposures to EDCs.

Key research gaps with specific reference to water testing and water quality for recognized water users within the EDC context highlight the difficulties in investigating the effects of EDC exposure water, but also present an opportunity.

This Manual is intended to not only direct research, but to also stimulate further research, with the objective of increasing the valid data required to formulate credible risk assessments, and in so-doing, be able to implement management actions based on informed judgments.

3 MONITORING AND ASSESSMENT

3.1 Sources, Pathways and Receptors

Source analysis may be thought of as Hazard Identification. It remains a central departure point in hazard and risk assessment to determine EDC exposure based on knowledge of the source of EDCs. For a hazard to be used in the context of a descriptive term that characterizes the intrinsic capability of an EDC to cause harm the EDC must first of all be present.

Source description needs to be performed sufficiently to be able to contribute to exposure-response relationships in both field and epidemiology investigations that can yield credible risk assessments. Exposure data needs to consider both historical and geographical trends over time, and current exposure values. This is particularly challenging as the fate and transport of existing chemical through various source media (water, sediment, and biota) are complex. In many cases the information used for reference purposes for source behaviour is obtained from chemical spills which are hard to extrapolate to low-concentration long-term source concerns.

To determine the possible routes of EDC exposure the behaviour of the EDC in terms of potential transport from the source to the receptor in question must be evaluated. This pathway analysis may be thought of as exposure assessment and also takes into account the actual level of intake involved.

It is also generally recognized that both the magnitude of exposure and relative potencies of multiple EDCs need to be considered. This implies that the context of exposure to an EDC cannot be only considered for one aspect of exposure, but needs to be seen in context of other sources as well. For human health this implies that EDCs in the environment need to be seen in conjunction with exposure to EDCs in the home and workplace. This also implies that different types of EDCs must be considered, for example, phytoestrogens and hazardous chemicals such as DDE, bisphenol A, and alkylphenols. This recognition of potency in terms of binding affinities for specific receptors assists in understanding the potential impact a source may have.

It follows that exposure assessment should also place emphasis on vulnerable groups that represent critical development stages (gestation, lactation, adolescence, and senescence). This is in part recognition that EDCs may affect cell proliferation, differentiation and organ development, hence exposure during sensitive periods provide the most significant potential for AEs. The type of receptor chosen determines receptor analysis and may be seen as the next logical step to determine the possibility for AEs following EDC exposure. This is becoming increasingly complicated by the general recognition that all routes of exposure need to be considered (dermal, inhalation, and ingestion) for multiple EDCs as the interactions may affect receptor events.

Numerous other challenges are present in EDC research, including rapid metabolism of some EDCs and the need to quantify metabolites in biological samples, transgenerational effects and the time lag between exposure and effect.

In the face of all these challenges and concerns it remains a common recognition that one of the most critical needs is for the continuation and improvement of monitoring of EDCs. The lack of data on trends in both environmental and tissue samples is largely due to intermittent observations which inevitably yield uncertainty.

In order for credible links to be made between EDC exposure and AEs, or the assessment of EDC exposure, monitoring provides a crucial fundamental platform from which assessments may be made. It is fundamental to link monitoring and assessment strategies as they are supportive. An indication of these links can be found in the stated objectives and design of the South African National Toxicity Monitoring Programme [28]. The stated objective of this programme is to measure, assess and report the status and trends of the nature and extent of potentially toxic substances in South African inland surface water systems, in order to support strategic management decisions. The next section introduces these concepts of Monitoring and Assessment.

3.2 Monitoring

3.2.1 General Concepts

In order to assess the potential effects of EDCs a number of specific factors need to be measured quantitatively. In broad terms the fundamental sequence of sources, pathways and receptors still applies. Although the act of monitoring can be defined in both general and more specific terms, the concepts of exposure and dose still apply.

This suggests that concentration and length of exposure are both relevant. Stated differently, data observing the occurrence of a chemical without sufficient detail on concentration variation over time and actual exposure periods in the organisms in which an AE is suspected or anticipated, yields less meaningful information.

The dual challenge with EDCs and AEs is that not only is the period of exposure involved typically very long (chronic, cumulative, and long-term), but the concentration is often very low, or at least, significantly lower than that required to produce classic symptoms of toxicity.

Further differentiation of type of effect is also required. Some EDCs may be considered to accumulate in specific body tissues and once at a critical or “threshold” level produce some undesirable effect. Others may only produce an AE after a cumulative exposure period, in which both historic exposure levels and exposure duration are as relevant as are current exposure observations.

The goal of monitoring may thus on some levels be considered as a proactive means of being able to accurately observe and interpret AEs.

An additional challenge is that exposure via a specific medium is often only an estimate. Furthermore, total exposure from all sources, whilst theoretically relevant, may be impractical to obtain. Models may be used to estimate the relevant combinations of exposure durations and exposure concentrations in order to arrive at a value for total exposure.

Indirect monitoring may utilize tissue concentrations to gauge or estimate these exposure concentrations and durations. This may be considered as biological monitoring in which the actual source and exposure time are not actually sampled and determined, but rather conclusions are drawn between values observed in specific biological samples.

A distinction is thus required between monitoring and biomonitoring. Biomonitoring is at times equated with residue analysis as this provides an indication of chemical dosimetry and body burden. A biomarker may be considered as a biological response obtainable from tissues, fluids (or even expired respiratory gases) as a measurable chemical, biochemical, physiological, cytological, morphological or any other appropriate biological parameter, that is observed for the purposes of discerning an association with exposure to an EDC.

This association is not necessarily direct, and in fact, may frequently be indirect. A biomarker may be used to obtain information regarding sources, pathways and receptors. As such they may assist with understanding the source (causative EDC), potential exposure, and thus possible predicted AEs. They may also be used comparatively between individuals, subgroups, or populations, in order to determine susceptibility, or more frequently, the outcome of site-specific factors that are too numerous and complicated to model, describe, or measure comprehensively.

An ideal biomarker may not necessarily be the most physiologically appropriate observation. This is as preference may be given for ease of sampling, number of available samples, and other factors, such as non-invasive procedures and cost of sample preparation and analysis.

General observations of tissue concentrations for a broad selection of chemicals are frequently employed in clinical biochemistry. Lowered detection limits, improvements in analytical procedures in terms of sample volume, preparation and capacity, and increased reference values for comparative norms, have led to this practice becoming more prevalent.

Biomarkers may further be considered in terms of the sample type, with sentinel species recognized as a valuable means for obtaining information from sensitive exposure models that assist in developing proactive responses to key user groups. For example, cattle have been used as sentinels

providing a range of biomarkers for indicating vanadium exposure and hence risk to human populations in an environment affected by industrial contamination [29].

The use of EDC concentrations in tissues is more typically employed for estimates of specific element toxicity diagnostics (and deficiencies). A range of clinical bands, from deficiency, marginality, adequacy to toxicity can be used for specific tissue types within not only species but also between breeds. These observations can allow for a progression towards targeted EDC-specific response investigations.

A summary of the general concepts is presented in Table 23.

Table 23. General concepts in monitoring.

Topic	Description
Quantitative measurement	Exposure considered for concentration (or level) and duration (time). The overall objective may be to quantify the relationship between exposure and health status. This needs to be considered with the type and frequency of monitoring carefully formulated.
Biological monitoring	Biological tissues may be selected to represent exposure and dose for a specific EDC. Sample selection may involve single or multiple tissue types. Both the time at which sample is collected and the actual types of sample collected depend on the chemical and type of adverse effect being investigated.
Sample variation	Water quality may be described as a stochastic variable (inherently variable). A single concentration may fail to accurately represent exposure so frequently a percentage of observations are required to exceed a set concentration value prior to prompting a sampling number increase.
Type of measurement	Measurements may be for individuals or groups. As individual measurements can vary greatly an increased frequency of observation may be required over a valid exposure time. As individual variation to the same dose occurs, a significant number of individuals need to be monitored in order to acquire valid data. Different methods may be used to interpret the distribution curves obtained. In some cancer studies a group or population dose may be used to reflect the assumption that for some exposure types all doses carry risk and that this risk increases linearly with that dose. A biostatistician is usually consulted during the formulation of a monitoring programme.

3.2.2 Specific EDC Monitoring Programs

In November 2009 the Water Research Foundation released a request for proposals for Endocrine Disrupting Compounds/Pharmaceuticals and Personal Care Products Strategic Initiative with an available per proposal fund of $400 000 and foundation funding commitment of $1.0 million/year directed specifically at monitoring tap exposures with water utilities targeted as the primary participants. This highlights the recognition of the importance of establishing EDC monitoring reference data.

A crucial point of departure in developing an EDC Monitoring program is to select the list of constituents to be monitored. As an example of how critical this step is, John and Trollip note in a report to the WRC in 2009 [30] that whilst the emphasis on developing standards for safe drinking water in South Africa has been on SANS 241 [26] compliance for the water quality constituents recognized as having a significant implication for human health, the actual chemicals used to treat water in order to make it safe are not subject to any control and they state that it "has been widely acknowledged and accepted by stakeholders that the continued use of such chemicals presents a high inherent risk from a public health point of view" .

This report also notes that whilst no current regulation exists in South Africa despite indications and clear concerns for the adverse effects linked to exposure to drinking water treatment chemicals the international trend is to stringently monitor and assure drinking water quality by legislative control of both production and use of these chemicals.

Although their report presents a chapter on risk assessment of drinking water treatment chemicals it does not include endocrine disruption as an effect for consideration as only Mineral Safety Data Sheets were used [30], highlighting the necessity to be clear regarding the nature and scope of the risk assessment performed. Although the report presents adverse effect concerns for 44 drinking water treatment chemicals, including those with published endocrine disruption effects (such as chlorine dioxide), no mention of such endocrine effects appears leading to potential misleading conclusions regarding the risk assessment conclusions.

Whilst it is recognized in literature that the interactions between chemicals are thought to be of significant importance when performing a risk assessment, this is not included in the report referred to [30]. Key interactions relating to chlorination methods and inherent inorganic water chemistry are the subject of research into EDCs as noted in the US EPA reference site http://www/epa.gov/scipoly/oscpendo/edsoverview/primer.htm. As such not only is it relevant to consider the water quality constituents capable of endocrine disruption and the drinking water treatment chemicals used to arrive to compliance quality, but it is also relevant to incorporate known interactions between these aspects that lead to the creation of constituents with endocrine disruption potential.

This challenge is well published regarding the replacement of chlorine with ozone as a drinking water treatment technology and the consequent need to monitor bromide concentrations due to the increased risk of unacceptably high main disinfection by-product formation, namely bromate, and subsequent carcinogenic risk [31].

An additional aspect that must be considered relates to the recognition that many monitoring programs are influenced by the condition of supply and not just quality issues. As such changing supply conditions, for instance during periods of drought, may lead to quality changes that are not always catered for that could result in significant disinfection by-product formation [32].

Monitoring and assessment are not only linked in a linear fashion as many assessments involve formulating risk management strategies, of which monitoring may form a main part. Monitoring programs may thus further place constituents into different categories based on the assessment process, for example, various programs within the Canadian legislature focus on monitoring category A or category B EDCs, defined respectively as pollutants with widespread distribution reported to have reproductive and endocrine-disrupting effects or reported to bind to hormone receptors and thus be suspected of these effects.

Other monitoring programs may focus on the desired end-result, for example a Toxic Substances Management Policy, in which some substances must be eliminated from the environment whilst others should be prevented or minimized from being released into the environment [33].

An unavoidable consideration in the development of specific EDC monitoring programs is cost and the ability to routinely handle the required amount of samples. Both cost and capacity considerations strongly influence the selection of constituents and inevitably influence the selection of priority compounds.

Lastly, the continual emergence of new compounds and substances with endocrine disrupting potential requires flexibility in updating the chemical candidate lists selected. In the interests of presenting a balanced perspective it is noteworthy that monitoring is also responsible for the removal of EDCs from monitoring programs, for example, the Endocrine Disruptor Screening Program of the US EPA deleted 6 of the original 73 chemicals on the list as monitoring revealed an absence thereof from three exposure pathways [34]. In some cases a separate list or term is used for many EDC substances, for example, the Constituents of Emerging Concern (CECs) used by the National Water Research Institute [35]. Although initiate in 1998 the US EPA Endocrine Disruptor Screening Program only produced a list of Tier 1 chemicals in April 2009, yet by November 2010 had already produced an updated second list.

In South Africa numerous water quality related monitoring programmes exist. These include the National Toxicity Monitoring Programme (NTMP), the National Aquatic Ecosystem Monitoring

Programme (NAEMP), and the National Radioactivity Monitoring Programme (NRMP), to name but a few, but it is recognized that the need exists for a "single version of the truth" to be presented regarding water quality [36].

It is also noteworthy that the South African National Toxicity Monitoring Programme states that the design and selection criteria had to be mindful of the financial and capacity constraints that exist, yet still be scientifically sound. Although this programme includes a recombinant yeast screen (hER) for oestrogenic and oestrogenic-mimics, some persistent organic pollutants (POPs) are excluded despite South Africa being a signatory to the Stockholm Convention due to lack of analytical capacity (furans and dioxins) [28].

In conclusion, as no standard monitoring programme for EDCs can be put forward that will satisfy all site-specific scenarios. A general framework is highlighted in Volume 5 that should allow for a standardized approach with existing methodology for catchment management. This challenge is highlighted in Section 4 (Case Studies) with the general approach regarding EDCs one which remains flexible and follows a stepwise approach to monitoring and assessment based on site-specific factors. As this Manual has water as the central focus some aspects pertaining to water quality monitoring are briefly dealt with next.

3.2.3 Water Quality Monitoring

Water quality monitoring is a complex field and is comprehensively presented in the scientific literature and in a wide variety of environmental and water-related institutions, and accordingly not repeated here. Some general observations relevant to EDCs are however highlighted here, with further detail noted in Section 4 (Case Studies). As a general observation it should be appreciated that water quality monitoring is understandably linked to the objective of the monitoring. Aquatic ecosystem investigations may therefore differ significantly in terms of the constituents selected and monitoring frequency from drinking water investigations for domestic purposes. It follows that EDC investigations may have a specific approach that can differ from other more established water quality monitoring programmes.

The challenge remains to link the various forms and purposes of water quality monitoring to provide meaningful data in order to:

- prevent unnecessary duplication
- allow for data collected to be of relevance to other assessments where possible
- allow for anticipated retrospective review of data to be conducted with sufficient observations and detection sensitivity

The design of a water quality monitoring is predominantly influenced by the water quality guidelines that are used to assess the monitoring data. In the case of EDCs this presents a significant challenge as the guidelines already in existence do not cater specifically for EDCs, but tend to be derived for other types of effects, for example, toxicological or carcinogenic. This topic is presented in more detail in Volume 5 of this Manual and should be regarded as an on-going developmental process. Although the EDC data types may be more appropriately linked to Hazard and Risk Assessment processes, they are nonetheless a form of assessment despite differing from current drinking water quality guidelines.

When reviewing international and local water quality guidelines for human drinking purposes as an example over the last two decades three common trends are noted:

- an increased number of WQCs listed to be able to assess water quality

- a decrease in the concentration limits considered acceptable

- the frequent revision and addition of constituents receiving global research attention

Without providing an exhaustive technical description other key problems are that guidelines tend to be formulated for local conditions with the WQCs listed largely a function of what is routinely detected in the local geochemistry and local laboratories. As many South African animal production systems rely on subterranean water it is essential to include a comprehensive constituent assessment as this is influenced by local geochemistry. In this regard international guidelines can be very misleading as they tend to focus on the constituents relevant to their particular set of local circumstances.

An additional problem is the delay in finalising new updated guidelines at a regulatory level whilst research continues to provide information that may suggest revisions are required. It is thus not surprising to observe the stated guideline limits to vary significantly between various published guidelines and recent scientific literature. An example of this may be found when comparing the 1996 Department of Water Affairs and Forestry (DWAF) guideline for selenium in domestic drinking water with the SANS 241 2006 and 2011 editions. The DWAF guideline set the target water quality range for selenium at 0.02 mg/L as did SANS 241 (2006) classifying <0.02 mg/L as the recommended operational limit, whilst the current SANS 241 (2011) has reduced this by 50% by setting an upper limit of 0.01 mg/L [2, 26, 37].

This change supports the trends referred to earlier and in accordance with the precautionary approach implies that monitoring should at take due regard for increased sensitivities required with the lowering of detection limits. It is noteworthy that the WHO Standards for Drinking Water observe that a factor in guideline derivation is the ability for laboratories to routinely detect the constituents at the concentration values suspected as being relevant. It also follows that a management value cannot be set at a concentration that cannot reasonably be achieved or determined.

Consequently, the specific water quality monitoring to be conducted may be formulated for a variety of different reasons and thus requirements, but as detailed in the Section 4 (Case Studies) inclusion of standard water quality tests that are currently available should form the fundamental basis for compliance monitoring.

3.3 Assessment

3.3.1 General Concepts

The goal behind monitoring and the assessment that follows is to develop an understanding of the key aspects pertaining to sources, pathways and receptors. This allows for a more accurate interpretation of exposure to EDCs and the potential for AEs to occur. This in turn allows for appropriate proactive measures to be taken to reduce the occurrence and/or severity of AEs. It is important to note that the AEs need not be completely prevented or removed, but in some cases comprehensively described and thus to contribute to the use thereof for monitoring and assessment purposes.

The use of the term “potential” implies sufficient biological plausibility that an effect following exposure may be adverse, even if the description of cause-and-effect or the precise mechanism is incomplete.

Some assessments are intended to describe the current situation and may form part of legal compliance or environmental audit requirement, or investigative research. Other assessments may be predictive in that they attempt to describe the potential for AEs used to plan and implement risk management strategies aimed at reducing (or potentially eliminating) risk.

The outputs of the assessment process assist with risk management. Usually the estimated risk is compared to an “acceptable risk” value or to other existing risks that are present.

The use of water quality guidelines is an example of this process and are intended to assist in reaching a decision on whether action is required or not. In general terms if the estimated health risk is too high then certain preventative actions may be implemented. Some of these actions may require increased monitoring, which may extend not just to the area or sample number but possibly to adjacent or wider areas of observation also.

For EDCs the situation is somewhat different due to the acceptance that the precautionary principle is valid. The terms “suspicion” or “suspected” may thus often be used in connection with EDC exposure and AEs, and this suspicion may be sufficient to warrant risk management actions.

Whilst the link between EDC exposure and endocrine disruption is being described the interest, from an assessment point of view, and a risk management perspective, is: Does the disruption suggest the estimated health risk is too high?

Risk management strategies can vary from exposure reduction, monitoring, targeted assessments, therapeutic intervention, to risk communication.

Both monitoring and assessment processes are usually revisited during risk management in the form of the monitoring of outcomes following the application of any measures or actions taken.

The concept of an assessment of risk may be taken to describe the chance of encountering the potential adverse effects from EDC exposure, this may be generally considered to represent the probability of harm or loss which is itself a product of probability and types of effects in terms of severity. This is discussed in more detail in Volume 5.

As risk is an integral component of life it is necessary to differentiate between Background Risk that is present independently of the presence of EDCs, and Incremental Risk that is due to the presence of EDCs. As an example, the background risk of cancer to the average U.S. citizen is 0.25 (one in four). If an EDC poses an incremental risk of 1×10^{-6} then the total risk is 0.25 plus 1×10^{-6}.

Assessments also investigate hazards, which are different from risks as they relate to descriptive terms that characterize the intrinsic capability of an EDC to cause harm. A hazard is in effect a source of risk and with regard to an EDC influenced by a variety of factors such as persistence, mobility, toxicity, and carcinogenicity.

The risk assessment process may be considered as:

- Hazard Identification (source analysis)
- Exposure Assessment (pathway analysis)
- Toxicity or Endocrine Disruptive Assessment (receptor analysis)
- Risk Characterization

As noted in the preceding section on EDCs the lack of appropriate data for all these steps provides significant challenges to performing assessments for EDCs. It is important to note that not all investigations need to complete this process. In other words the objective may not always be to perform a risk assessment. Each of these steps in the risk assessment process contribute to our understanding of EDCs and AEs and, in conjunction with the precautionary principle and expert opinion based on accepted scientific evidence, may be used to formulate corrective measures, guidelines, and/or actions that relate in principle to risk management.

3.3.2 Uncertainties

Uncertainties in risk assessment occur at all levels of the process and are an inherent part of the process. Whilst it may be accepted that given the current knowledge available accurate assessments on AEs following exposure to EDCs may not be adequately predicted, these methods may still be

used to rank and compare EDCs and exposure scenarios. This still allows for a basis whereby required actions may be prioritized.

It is important to note that this challenge is not unique to EDCs, and that both classic toxicity and carcinogenicity risk assessments are based on assumptions and extrapolations which result in uncertainties.

These uncertainties may be further described as:

- Source uncertainty:
  - Inaccurate sampling
  - Analytical detection limitations
  - Improper EDC selection

- Uncertainty in data:
  - Lack of required source data
  - Lack of reference doses (for toxicity) and slope factors (carcinogenicity)

- Assessment models and methods:
  - Uncertainty in predictive models is inevitable
  - Uncertainty in sampling data is also possible (e.g. false positive and false negatives)

- Reference Data:
  - Extrapolation uncertainty due to use of models (toxicological data using animals for human toxicity; groundwater-aquifer contamination models)
  - High variability in safety factor chosen
  - Poor comparison between predictive models and low-probability events
  - Practice of adopting conservative estimates to address uncertainties may limit interpretations of exposure and effect observations
  - Study design often not suited to determine causation

Assessment is required to meaningfully interpret monitoring data. As an illustration of this a fact sheet on EDCs and Waste Water Treatment published by the Water Environment Research Foundation (www.werf.org fact sheet 04WEB6a) notes that although due to laboratory technologies currently available EDCs have been detected virtually everywhere, no study clearly demonstrates a link to suspected EDC effects and human health based on exposure to lakes, rivers and streams. It continues to note that even though some adverse effects have been observed the results may also be attributed to other causes and not just EDCs and that the risk assessment process is required to determine if these other factors may be causative or not. An example of this stance is to be found in work published regarding exposure to 17-alpha-ethinyestradiol, a key component in birth control pills,

which concludes that the risk to human health from exposure is negligible [38]. Whilst this may seem at odds with the statements release by the Endocrine Society noted in section 2 the fact sheet draws attention to the disparity often noted in published work regarding the presence and effects of EDCs. It also concedes that many issues are not yet understood, for example, the EDC effects of biosolid disposal on land and environmental consequences of recycled water, and furthermore states that more research is needed to fully understand the risks.

Just as monitoring alone is not sufficient to describe risk following exposure the assessment process also has many limitations, one of which is that the predicted effects are in many instances difficult to prove or even monitor. A fundamental aspect in research findings concerning EDC exposure and effects is that if no adverse effect is observed then it is assumed that the research failed to find an association between the exposure and an effect. It is thus concluded that no adverse effect was observed but it is not concluded that the effect did not occur (absence of effect). On-going studies are thus required to build confidence (certainty) in arriving at the statement that an exposure-effect relationship does not exist at a significant level, but single studies failing to observe an effect caused by exposure cannot as a consequence arrive at the conclusion that such an effect did not occur (uncertainty in the absence of an effect).

An example of the decision making process sequence may be provided by the review of pesticide registration by the US EPA in which several legislative and collaborative efforts from different interested and affected parties are involved. Use of a pesticide is governed by the Federal Insecticide, Fungicide and Rodenticide Act (or 1947) and should a review be called for based on monitoring data and subsequent assessments that indicate either unacceptably high risk to humans, animals, non-target organisms, and the environment, then the US EPA can choose to either take no-action or some forms of action to reduce the risk. Examples of these actions may include re-labeling, reclassify approved usages, suspend some uses, suspend the registration or cancel the registration.

Given that an estimated 1 in 35000 chemicals pass initial testing to succeed in market placement and approval requires several years and over 140 test, the evidence gathered from monitoring and the accuracy of the assessment process are both required to be of a high standard and thus be sufficiently scientifically defensible.

It is also noteworthy to take cognizance of the vast number of potential interested parties and organizations involved. For example, the testing of pesticide residues of imported products may fall under a different authority (e.g. Animal and Plant Health Inspection Services of the US Department of Agriculture) to that responsible for locally produced food (e.g. US EPA). More than one organization may also have monitoring programs in place (e.g. US Food and Drug Administration may test pesticide residues in fruit and vegetables) [24]. These programs are also often linked to international efforts, such as the Codex Alimentarius Commission which links the US EPA to the United Nations Food and Agriculture Organization and World Health Organization.

It is important to note that with continued concern regarding exposure to EDCs a key criticism of these various monitoring and assessment programs that lead to initial and on-going registration relates to the amount (dose) considered in the assessment process and the lack of exposure to “mixtures” of potential EDCs. As noted in the Scientific Statement released by the Endocrine Society of the US concerns relate to very low-dose exposures to multiple potential EDCs [8].

Some of these concerns are addressed by the application of site-specific hazard and risk-assessment procedures that investigate not only the potential of exposure but the combination of multiple exposures and final outcomes of risk factors present.

3.3.3 Assessment Communication

No monitoring and assessment program should be undertaken without a clear understanding of the type of information to be produced and the manner in which communication of the findings will be performed.

Whilst this topic is covered in more detail in Volume 5 of this Manual and numerous reference texts are more suited to cover the relevant complexities it is necessary to introduce the concept of “acceptable risk”.

This topic is a fundamental aspect of EDC research as it continues to emerge that animals and humans are unlikely to not be exposed to EDCs. As such risk cannot be completely removed and “zero” risk can thus not be achieved. This concept is arguably adopted by the general public on a selective basis, for instance in the use of public transport systems and commercial travel, there is often a strong objection to the presence of risk associated with some types of exposure irrespective of risk level. Drinking water, medical procedures and food quality are some topics where the general public seeks or prefers a “zero” risk level.

To illustrate this the Water Research Foundation, the leading research organization in the USA regarding drinking water, stated at a congressional hearing in February 2009 that “Even the most advanced treatment processes that we’ve studied won’t achieve an absolute zero level of contaminants”. Consequently the organization allocates a large portion of its resources to assessing the risks posed [39].

A key statement released in 2009 by this organization following a three year investigation in collaboration with 17 water utilities stated that “The concentrations of pharmaceutical drugs and endocrine-disrupting compounds found in our public drinking water are likely too low to impact human health” [39]. It is notable how contradictory this statement is to the concerns expressed in the same year by the Endocrine Society of the United States [8].

When reviewing the methods employed and data obtained from this report two key factors emerge. The first relates to the "monitoring" component in the form of the representation limitations of the data on which the statement is based. The assessment was based on a total of only 300 water samples which although seemingly sufficient represents a fraction of available public water supplies. Only 62 compounds were selected for testing and a glance at Tables 13 to 16 illustrates the vast number of potential EDCs currently recognized. Some of the results are presented in a specific manner that may be interpreted by some to be a "selective" representation of true risk. Some of the findings presented were: three compounds detected in <50% of the samples; 11 compounds detected at "trace" concentrations in finished (treated) drinking water; 5 prescription pharmaceutical drugs (atenolol, Dilantin, carbamazepine, gemfibrozil and sulfamethoxazol); "trace" concentrations of atrazine (pesticide), DEET (insect repellant), metolachlor (pesticide), two flame-retardants, Tris (2-chloroethyl) phosphate, and Tris (chloroisopropyl) phosphate.

The second factor relates to the "assessment" aspects. Whilst the highest levels of atrazine detected were 870 ng/L and the regulatory limit is 3000 ng/L this limit does not, in the view of the Endocrine Society of the United States, accord with recognition for low-dose effects and exposure to multiple EDCs [8]. The report states that although "Concerns may be raised because detection…seems to be evidence enough of risk", the context of risk is acceptably low as "..in the world of toxicology, it's the dose that can create a health risk. It's the concentration that matters.".

Both these statements are at the very least misleading and arguably totally incorrect with regards to EDC exposure as EDC effects do not reside in the realm of classic toxicology (see section 1) with dose-response curves regarded by specialists to be insufficient in estimating endocrine-disruption and the assessment statement does not account of for simultaneous exposure to multiple EDCs, despite the recognition in the statement that this in fact occurs. Numerous other considerations presented in section 2.7 were not incorporated in the assessment and this report is thus referred to as an example of "selective" reporting of monitoring and assessment data and is indirectly referred to in the Scientific Statement release later that same year by the Endocrine Society of the United States [8].

The topic of risk communication with specific reference to water quality is also presented in a separate WRC Project (TT 298/07) and the reader is referred thereto for further detail [40].

3.4 Health Risk Assessment of EDCs in Water

3.4.1 Background

Health risk assessment is the process or method of determining if an activity (man-made or natural) will negatively impact humans. Risk assessments are a prerequisite for the development of policy and legislation to protect humans and the environment from hazardous substances. These assessments are therefore used as a decision making tool in guideline and management strategies, to support decisions that protect public and environmental health.

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 [41] and involves four distinct, but interacting phases [42], namely:

- *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

- *Dose-Response Assessment* characterises the relationship between the dose of a hazardous agent 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.

- *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.

Although endocrine disrupting compounds cause serious concerns, there is no standardised method or guideline to assess human health risks associated with endocrine disrupting chemicals [43]. Current human health risk assessments differentiate between risks from chemical substances that cause carcinogenic (causing cancer) or toxic (non-carcinogenic) effects [44].

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 [45, 43]. 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 [45, 43]. 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. According to the World Health Organisation, the health risk assessment approach is the recommended process used to derive guideline values for substances in water [43]. 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. Conventional toxicology assumes 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 [46, 47].

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) [47] 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 [48]. When following a U-shaped response curve, the strongest responses are found at low and high concentrations [44]. 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 [49, 47, 44].

3.4.2 Recent Developments to Human and Wildlife Exposures to EDCs

In 2002, the International Programme on Chemical Safety (IPCS), a joint programme of the World Health Organization (WHO), the United Nations Environment Programme (UNEP) and the International Labour Organization, published a document entitled Global Assessment of the State-of-the-Science of Endocrine Disruptors [9]. This work concluded that scientific knowledge at that time provided evidence that certain effects observed in wildlife can be attributed to chemicals that function as endocrine disrupting chemicals (EDCs); that the evidence of a causal link was weak in most cases and that most effects had been observed in areas where chemical contamination was high; and that experimental data supported this conclusion. The document further concluded that there was only weak evidence for endocrine-related effects in humans.

Now, in 2012, the United Nations Environment Programme (UNEP) and WHO present an update of the IPCS (2002) document, entitled State of the Science of Endocrine Disrupting Chemicals—2012 [50].

There is far more knowledge on exposure to EDCs and potential EDCs today compared with 10 years ago (State of the Science on EDCs, 2012). Most of the latest findings and conclusions have been summarised in the State of the Science on Endocrine chemicals report (2012) put together by the WHO and US EPA. Key findings were;

- Humans and wildlife are exposed to far more EDCs than persistent organic pollutants (POPs) of which only a fraction of EDCs in the environment are currently known.

- Endocrine systems are very similar across vertebrate species and that endocrine effects manifest themselves independently of species. Therefore the effects of EDCs are endocrine system related and not necessarily species dependent. Effects shown in wildlife or experimental animals may also occur in humans if they are exposed to EDCs at a vulnerable

time and at concentrations leading to alterations of endocrine regulation. Of special concern are effects on early development of both humans and wildlife, as these effects are often irreversible and may not become evident until later in life.

- Humans and wildlife are exposed to multiple EDCs at the same time, and there is justifiable concern that different EDCs can act together and result in an increased risk of adverse effects on human and wildlife health. A focus on linking one EDC to one disease severely underestimates the disease risk from mixtures of EDCs. We know that humans and wildlife are simultaneously exposed to many EDCs; thus, the measurement of the linkage between exposure to mixtures of EDCs and disease or dysfunction is more physiologically relevant. In addition, it is likely that exposure to a single EDC may cause disease syndromes or multiple diseases, an area that has not been adequately studied.

- Internationally agreed and validated test methods for the identification of endocrine disruptors capture only a limited range of the known spectrum of endocrine disrupting effects. This increases the likelihood that harmful effects in humans and wildlife are being overlooked. More comprehensive assessments of human and wildlife exposures to diverse mixtures of EDCs are needed. For many endocrine disrupting effects, agreed and validated test methods do not exist, although scientific tools and laboratory methods are available. For a large range of human health effects, such as female reproductive disorders and hormonal cancers, there are no viable laboratory models. This seriously hampers progress in understanding the full scale of risks. Ideally, a highly detailed map of environmental exposures that might occur throughout a lifetime should be developed.

- Exposures to EDCs occur during vulnerable periods of development, i.e. fertilization, foetal development, nursing of offspring etc. which raises particular concern.

- New sources of exposure to EDCs have been identified. These include indoor environments and electronics recycling and dumpsites. The speed with which the increases in disease incidence have occurred in recent decades rules out genetic factors as the sole plausible explanation. Not all sources of exposure to EDCs are known because of a lack of chemical constituent declarations for materials and goods.

- Spatial and temporal monitoring is critical for understanding trends and levels of exposure.

- Levels of EDCs in humans and wildlife are related to how much a chemical is used. Bans on several POPs have led to declines in environmental levels and human body burdens. In contrast, there are increasing levels of some newer EDCs, such as perfluorinated alkyl compounds and replacements for banned brominated flame retardants.

- There is global transport of EDCs through natural processes (ocean and air currents) as well as man-made activities, leading to worldwide exposure of humans and wildlife to EDCs.

- Despite substantial advances in our understanding of EDCs, uncertainties and knowledge gaps still exist. An integrated, coordinated international effort is needed to define the role of EDCs in current declines in human and wildlife health and in wildlife populations.

3.4.3 Effectiveness of Current Risk Assessment Methodology for EDCs

The major question is; is there enough information or conceptual understanding of endocrine disruptors in order to plan action in health risk assessments? Amidst the uncertainty there have been world-wide urgent calls to address the possible and seemingly increasing health impacts caused by endocrine disruptors. Internationally, this has inevitably led to the adoption of simplified models with which to devise assays and hazard definition /risk assessment methodologies. These models and prediction contain inherent compromises, which will need to stay open to the impacts of new insights and updated laboratory findings [51].

Usually the studies used to derive a 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 [43].

The properties of EDCs which influence the current methodology to assess the human health risks associated with them are discussed in a previous report (WRC KV206/8) [67] and can be briefly summarised as:

a) 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 [52]. It is difficult to establish dose-response relationships for human exposure of EDCs and incident disease, as most people have been exposed to some form of EDCs somewhere in their lifetime, leaving a very small possibility for a controlled reference group [51].

b) Threshold and linear model assumptions – As already mentioned, 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.

c) Some EDCs display transgenerational properties can be transferred across the placenta and into maternal milk, thereby affecting the foetal development and resulting offspring [44]. 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 [53]. Developmental effects of endocrine disruptors tend to be latent, where traditional endpoints of toxicity may not be detectable until sexual maturity [54].

d) EDCs are mostly tested and evaluated in adult animals, although some EDCs are known to affect test groups in early developmental stages, in addition, the effects of exposure during early life stages may not manifest until adulthood [55]. Timing of exposure or delayed effects is therefore crucial in assessment procedures.

e) Generally, toxicological studies examine the effects of only a single chemical at a time. EDCs can act additively and even synergistically [56, 57, 58]. Evidence exists that simultaneous exposure to multiple EDCs may result in a combined response more than the threshold for effects, even though individually each chemical is below its effect level [59]. Therefore chemicals that have been classified as safe, may still pose harmful effects when combined with other EDCs [53, 44].

f) Evidence of effects and correct end-points is essential when conducting the dose-response assessment of a chemical thought to be an EDC [60, 61]. 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 [63].

3.4.4 EDC Prioritization

The first step that should be considered in any assessment is the availability and accuracy of available information. Monitoring can prove a time consuming and expensive exercise and would benefit from a prioritization system, is which target molecules for assessment can be identified after a screening exercise. Thereafter effective monitoring and assessment can commence for chemicals with strong evidence of endocrine activity.

A priority list of chemicals was developed within the EU strategy for endocrine disruptors. This list was established in two phases:

1) Independent review of evidence of endocrine disrupting effects and human/wildlife exposure

2) A priority setting exercise in consulting with stakeholders and the Commission of Scientific Committees (including SCCS, SCHER, SCENIHR and ICCG).

The priority list was drafted into a database and is constantly undated with evidence based studies. The EU EDC prioritization followed a four step process:

Step 1:

A working list of the suspected EDCs surveyed in the literature was compiled. This was supplemented by any and all data available on toxicity studies conducted with these chemicals.

Step 2:

The available information was again reviewed to determine the persistence of these chemicals as well as the volume of the chemical added to the environment through anthropogenic discharges.

Step 3:

Chemicals with high persistence and high volume input were reviewed to determine the strength of evidence for endocrine disruptive behaviour. These chemical were then divided into 3 categories:

- Category 1 – evidence of endocrine disrupting activity in at least one species using intact animals
- Category 2 – at least some in vitro evidence of biological activity related to endocrine disruption
- Category 3 – no evidence of endocrine activity or no data available

Step 4:

Literature on Category 1 chemicals would be reviewed to determine the likelihood of human or wildlife exposure. Highest priority would then be given to chemicals which humans and wildlife and most likely exposed to at elevated concentrations.

Close to 800 chemicals are known or suspected to be capable of interfering with hormone receptors, hormone synthesis or hormone conversion based on their chemical structure and binding characteristics. A total of 564 chemicals have been either published or reported by various organisations as being suspected EDCs. Of these, 147 compounds are considered persistent or are produced at high volumes. Clear evidence of endocrine disruptive behaviour was noted for 66 chemicals (Category 1) and some evidence was reported for 52 chemicals (Category 2). Humans are considered to be likely exposed to 60 of the 66 Category 1 identified EDCs.

3.4.5 Quantitative Risk Assessment

3.4.5.1 Hazard Identification

Chemicals pose a variety of health effects that are reviewed in detail in EPA's Integrated Risk Information System (IRIS) Toxicology Reviews, the Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles, the World Health Organization's International Programme for Chemical Safety (WHO/IPCS) Environmental Health Criteria Documents, and metal toxicology reviews.

3.4.5.2 Dose Response

The hazard is further characterised by a dose response. Herein the amount of potentially harmful chemical likely to cause an adverse health effect in an exposed population is described. These potentially toxic doses are determined from many laboratory and field toxicology studies as well as reported incidences of human and animal exposure. International organizations such as the WHO and USEPA then summarised and reviewed reported studies in order to

- developed guidance values that are based on toxicological and epidemiological information, such as the allowable daily intake (ADI) and total daily intake (TDI), which provide an estimate of the amount of chemical that can be taken in orally by a person without appreciable health risk ; and

- quality guideline values for chemical concentrations in drinking-water, air and food (the exposure medium). Based on ADIs and TDIs, these values usually take into account multimedia exposure scenarios (e.g. the WHO Guidelines for drinking-water quality) or are based on agricultural practices and climate).

This information can (generally) be very simply accessed by health risk assessors through established databases such as IRIS and ATSDR established by the USEPA and WHO.

3.4.5.3 Exposure

Generally the assessment of exposure to contaminated water is carried out as follows:

- Identifying how people come into contact with chemicals in the environment

- Identifying the pertinent exposure metric (e.g., oral intake, inhalation exposure concentration, blood concentration).

- Identifying sources of uncertainty and natural variability and, where possible, quantifying these in estimates of exposure

- Determining the concentration of contaminant in intake media (monitoring data)

- Given reasonably information on the target community, determine the rate of intake. The determination of reasonable average consumption rates is usually a complex task, because human food patterns vary significantly from place to place. The life stages, dietary aspects and exposure pathways will be unique and integral to a meaningful health risk assessment. If this information cannot be sourced from a community, default values are provided by the USEPA's Exposure Factors Handbook [63].

- Finally, a dose is derived which is taken forward to the risk characterisation.

3.4.5.4 Risk Characterization

For non-carcinogenic toxic effects of heavy metals, a Hazard Quotient (HQ) is calculated, comparing the expected exposure to the agent to an exposure that is assumed not to be associated with toxic effects. For the oral exposure of humans to the consumption of contaminated water and plants, the Average Daily Dose (ADD), determined from the measured concentrations, was compared to a Reference Dose (RfD) as reported by the US EPA.

A non-carcinogenic risk HQ <1 is considered to be safe for a lifetime of exposure. For cancer assessment, risk is expressed as a risk per unit dose in which 1 E-06 (1 in 1000 000) is considered safe for a lifetime of exposure. Each risk characterization should include three components: a qualitative summary of each section of the risk assessment, a numerical risk estimate, and a description of uncertainties.

3.4.6 Bioassays for Endocrine Disruption Activity in Water

A biological assay (bioassay) is a procedure which uses the responses of living organisms, or components of their biological system, as an analytical tool to detect;

a) the presence of response altering chemicals

b) the effects of these chemicals

c) the potency of these chemicals in comparison to a reference material [64].

Bioassays can employ entire biological systems or components thereof at all levels of biological organization (molecules, cell, tissue etc.).

Biological methods provide valuable screening tools due to the unknown specific chemical nature of an environmental sample. 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 disruptors in water is the number of possible chemical contaminants responsible as well as the complexity of the tests.

Bioassays are conducted in one of two ways; a) *In vivo* bioassays use living organisms, such as animals, while b) *In vitro* bioassays exploit the responses of components of organisms (e.g., tissues, cells, receptors) maintained artificially in a laboratory in sterile conditions.

The majority of biologically based methods, particularly *in vitro* bioassays, for EDC determination in water, are intended for oestrogenic or anti-oestrogenic chemical effects. Commercial bioassays for EDCs that act on the thyroid have not yet become available [64].

In vitro responses are quicker, simpler to conduct, less expensive and therefore more ideal as screening tools. *In vitro*-bioassays for EDCs are based on relatively well characterised modes of actions (MOAs) and have easy to interpret endpoints. However, these bioassays have limited metabolic capabilities and have potential for false negative responses. *In vitro* bioassays are typically less biologically relevant compared to results from *in vivo* bioassays and therefore have comparatively less scope in assessing human health risks [65].

In vivo bioassays for EDCs may be time consuming and costly, may in some cases lack sensitivity and produce modest responsiveness. These bioassays are however, more useful for assessing the potential for a substance to act through any of a number of possible MOAs to cause a particular effect. *In vivo* studies compare the biological responses of exposed living organisms to that of a reference organism of the same specie contained within a controlled environment. The established *in vivo* bioassays widely used by the European Organisation for Economic co-operation and development (OECD) or other national guidelines [50] are briefly described in Table 24.

Table 24: Established *in vivo* bioassays which have been in wide use as validated OECD or test guidelines (adapted from USEPA, 2012) [50].

Bioassay	Animal subject	Description
Amphibian metamorphosis (OECD TG 231)	tadpoles	Thyroid disruption leading to developmental effects
Hershberger (OECD TG 441)	mammals	Detect androgenic and anti-androgenic effects. Sex glands of several androgen-dependent tissues are weighed. Histopathologic changes in thyroid. Changes in serum T4 and T3.
Pubertal Development and Thyroid function assay Female / Male (US EPA OPPTS 890.1500 and 890.1450)	mammals	Screen for oestrogenic, androgenic and thyroid activity. Examines abnormalities associated with sex organs and puberty. Histopathologic changes in thyroid (follicular cell height increase & colloid area decrease). Possible effects on preputial separation.
Uterotrophic (OECD TG 440)	Mammals	Screen for oestrogenic and androgenic effects. Measure of uterine weight.
Amphibian/ Mammalian /Avian 2 – Generation (OECD TG 416)	Birds; mammals and frogs	Estrogenic and androgenic activity disruption. Effects on vaginal opening; oestrus cyclicity and preputial separation. Increased thyroid weight. Histopathologic changes in thyroid. Possible effects on fertility.
Fish Lifecycle Toxicity Test (FLCTT) (US EPA OPPTS 850.1500)	Fish	Measures male and female biased phenotypic sex ratio endpoints. Altered levels of thyroid hormones. Possible effects on VTG depression.
Extended 1 – Generation reproductive Toxicity study (OECD TG 443)	Mammalian, fish	Oestrogenic and androgenic effects. Genital abnormalities. Increased thyroid weight. Histopathologic changes to thyroid.
Fish short term reproduction assay (FSTRA) (OECD TG 229)	Fish	Oestrogenic and androgenic effects. VTG depression in females. Depression of 2nd generation sex characterization
21-Day Fish Assay (OECD TG 230)	Fish	Estrogenic and androgenic effects. VTG depression in females. Depression of 2nd generation sex characterization.
Androgenised female Stickleback Screen (AFSS)(OECD GD 140)		Androgenic effects. Spiggin induction/ depression.
Fish Sexual Development Test (FSDT) (OECD TG 234)	Fish	Oestrogenic and androgenic effects. VT induction in both males and females. Male and female biased phenotypic sex ratio.
Avian Reproduction Test (OECD TG 206)	Avian	(no end-point EDC included) Egg production, cracked eggs, eggshell thickness, egg viability.

In order to assess whether endocrine disruptors are present in water one can do one of two things:

- Carry 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 first option is usually 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.

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.

Bioassays selected for the detection of EDCs in source water and characterisation of their potential to cause health effects should be carefully researched to identify both strengths and weaknesses prior to implementation, and the meaning and importance of bioassay results should not be extrapolated beyond the intended purposes and capabilities of the bioassay [64].

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) [10, 12]. Therefore, the need exists to develop a recommendation for a suite of suitable and reliable methods available for conducting this analysis.

3.4.7 The Trigger Value Approach

3.4.7.1 Background

Human health risk assessments are flawed by their inability to deal with multiple chemical exposures when deriving meaningful human health effect predictions. With the multitude of disruptive pathways that endocrine disruptors can follow, and the impacts that several EDCs can have on each other as well as having opposing disruptive pathways, it is evident that it is impossible to derive an over-all guidance value for maximum permissible oestrogenic activity. From here a trigger value is suggested. This trigger value is based on various findings and assumptions made during human health risk

assessments and is 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 www.nusap.net).

A Framework for EDCs guidelines for drinking water in South Africa has previously been suggested [66]. The framework used a tiered approach in which water samples were first screened reproductive endocrine disruption capability as oppose to individual chemical evaluation. The suggested approach involved the use of a trigger value of oestrogenic activity using bio-assays. A battery of in vitro and in vivo tests was recommended to quantitatively express the results of oestrogen activity of a water sample containing a mixture of chemicals in terms of their relative potency.

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 trigger value was based on a WHO 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.

3.4.7.2 Calculation of equivalent trigger value

Calculation of the trigger value has been extensively reported in WRC K206/08 (2008) and again briefly summarised here. The equation to calculate the trigger value is as follows:

$$Guideline\ value = \frac{ADI * bw * P}{IR}$$

Where:
ADI = acceptable daily intake;
bw = body weight;
P = portion of exposure allocated to water;
IR = intake rate of water

Based on the proposed risk assessment framework to derive guideline values, the trigger value is based on the acceptable daily intake (ADI). The ADI for 17β-oestradiol has been calculated by the WHO as 50 ng/kg/d, based on induction of hormone changes in post-menopausal women who have low endogenous oestradiol production. 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. In the case of 17 β -oestradiol the uncertainty factor used was 1000 (RIVM, 2004 www.nusap.net).

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.

Most water quality guidelines use 10% as an allocation to account for additional exposure through other routes (e.g. inhalation and dermal absorption). The trigger value was determined to be 0.7 ng/L based on the consumption of 2 L of water, assuming an average body weight of 65 kg and a exposure portion of 0.1 %. Monto Carlo simulations were conducted to account for variation in body weight and daily intake [66].

3.4.7.3 What to do if the Trigger Value for Drinking Water is Exceeded

The following steps are recommended to be taken if the trigger value of 0.7 ng EEQ/ is exceeded in the screening test;

a) Ensure that sampling was done in duplicate and that samples were evaluated according to identical methodology.
b) Ensure that all SOPs and good laboratory practices have been adhered to
c) Repeat analysis
d) Assemble a team to ascertain the possible sources/ causes of contamination.
e) Check if all water treatment processes and operations are functioning optimally.
f) If all processes are found to be working optimally, a catchment to tap assessment should be conducted.
g) Targeted chemical analysis should then be done to assess which chemical or group of chemicals are responsible for the oestrogenic activity.
h) Routine monitoring for oestrogen activity should continue. It should always be considered that the trigger value is not a guideline value. Guideline values are set by water administrators after sufficient scientific evidence is presented.
i) Communicate risk to the community according to a strategically well devised health risk communication plan made according to the procedures described in Genthe and Knoetze, (2008) [40]. Health risk communication cannot just be the provision of scientific evidence for a possible health risk. The objectives of constructive and well managed risk communication are to inform the public of risks, understand the significance of potential risks and to manage perceptions.
j) After briefing a community of possible health risks, supporting evidence for endocrine activity can be gained in collaboration with the community and health risk investigators by an *in vivo* bioassay using sentinel specie. If the resources are available, i.e. a possible local reference sentinel in a controlled environment, reproductive and thyroid-related developmental effects may be investigated.

3.4.8 Methodology

A methodology is suggested to assess the possible endocrine disrupting effects of chemicals on human health. Herein, an assessor is led by key questions and outcomes expected as suggested by the step modified from the Risk Assessment Toolkit developed by the WHO [68].

Hazard identification

- *What is the identity of the chemicals of concern? Is endocrine activity suspected*? It is most likely that a multitude of chemicals and their derivatives would occur in a given water sample. These chemicals may increase or negate the endocrine effects of other chemicals in a given mixture. These impacts may be evaluated according to the WHO [68], although very few studies exist on resulting toxic effects of these mixtures. To be conservative in risk estimation, chemicals are therefore generally evaluated as if occurring in isolation in a given water sample. The calculated risk hazards posed by individual compounds are summed together for a single water sample.

- *Are the chemicals potentially hazardous to humans*? This is determined through review of given Toxicology databases. In the case of heavy metal, reported toxicity evaluations are generally specie specific. Given that the most cost effective method for metal evaluation is total metal determination, an assessment simplification is the assumption that the total reported metal concentration occurs in the ionic (salt) form in a given water sample.

- *What properties of the chemicals have the potential to cause adverse endocrine effects*? As stated in the background study, specific endocrine evaluations have only recently been included in toxicology evaluations. Much of the possible endocrine activity caused by certain compounds can be observed in specific laboratory studies. Therefore toxicological studies will need to be reviewed to determine if any endocrine effects can be expected for a given compound. Herein the dose response including reference dose (RfD), acceptable daily intake (ADI) etc. (if any) for the suspected ED are considered.

- *Do health-based guidance or guideline values from international organizations exist for the chemicals of concern?* This will determine whether quantitative risk assessment for the chemical compounds is possible.

- *What assumptions about exposure and dose are incorporated into the WHO drinking-water guideline values (or other national authorization) for the compounds of concern*? The assumptions include the body weight of the average person exposed, the frequency of consumption, likelihood of consumption etc. These values can either be determined by communication with authorities or community of people exposed to water contaminated with the chemicals of concern. If information cannot be sources, default values set by the WHO can be used.

- *Do these assumptions reflect the conditions specific to the given case study*? Are the guidelines appropriate? Many South African communities are perceived to be more vulnerable to the effect of certain contaminants, due to impoverished living conditions and limited access to basic services. Stricter guidelines can therefore be considered to protect vulnerable communities.

A (generic) summary of the steps toward hazard characterization is given in Figure 1.

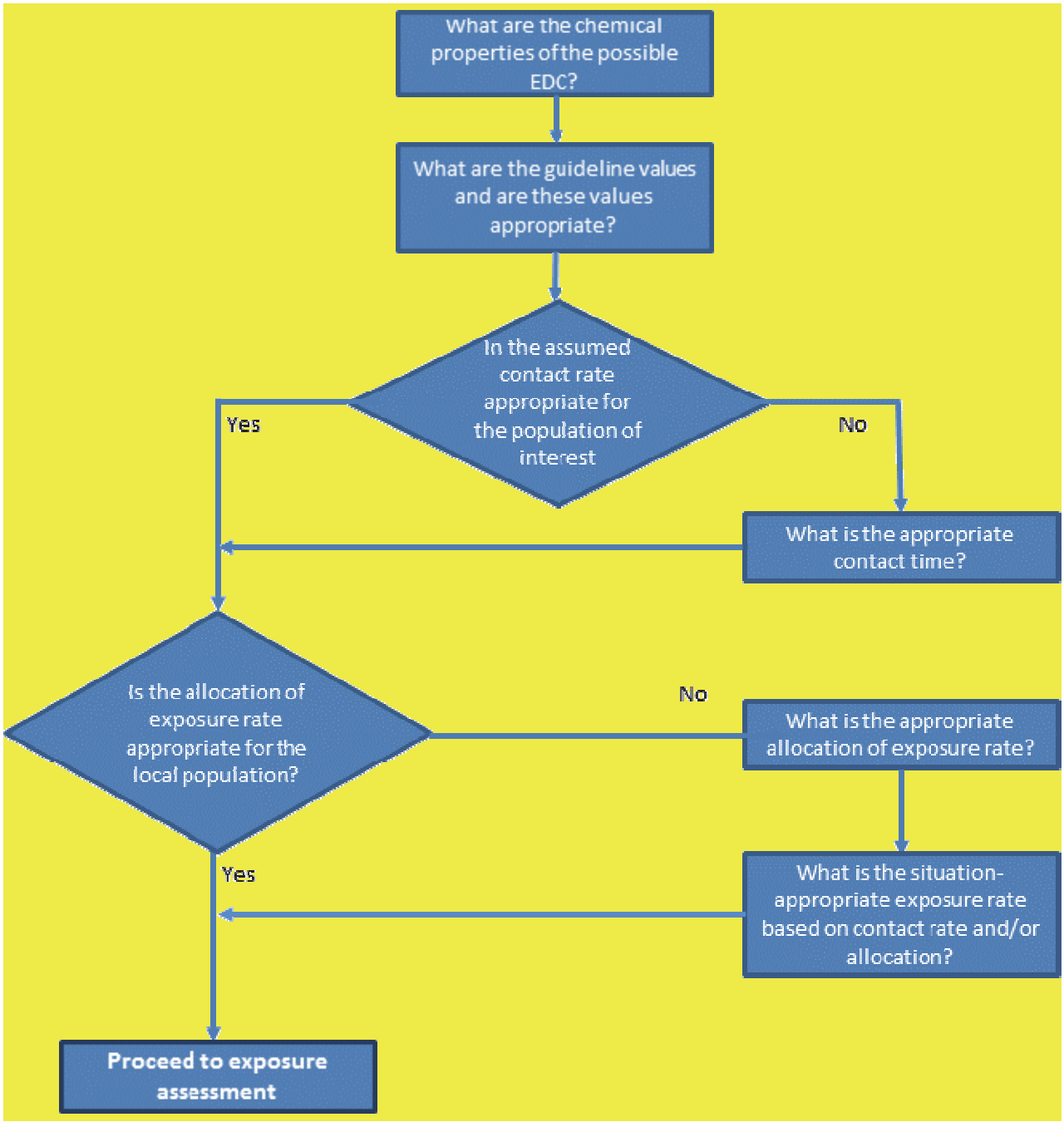


Figure 1: A generic road map for hazard characterization [68].

Exposure assessment

- *In which ways are people exposed to the chemicals of concern?* For the current guideline recommendations, exposure to chemical compounds through the consumption of water is considered.

- *How much exposure is likely to occur?* Quantitative assessment of the amount of chemicals the community/ people are exposed to. Chemical concentrations are determined in water samples by accredited laboratories and approved standardized methods. From the measured chemical concentrations, the average daily dose (ADD) people are exposed to, is determined. This requires information on the daily intake and body weight of the persons exposed.

- *How long is exposure likely to occur*? This will give an indication on whether short or long term exposure should be considered. Long term exposure is calculated by determining a lifetime average daily dose (LADD) (Table 24) and is used in carcinogenic evaluations.

- *How much endocrine activity is observed for a given water sample?* Submitting a sample to assess the biological activity will determine a subset of endocrine activity. A summarization of the exposure assessment steps is given in Figure 2.

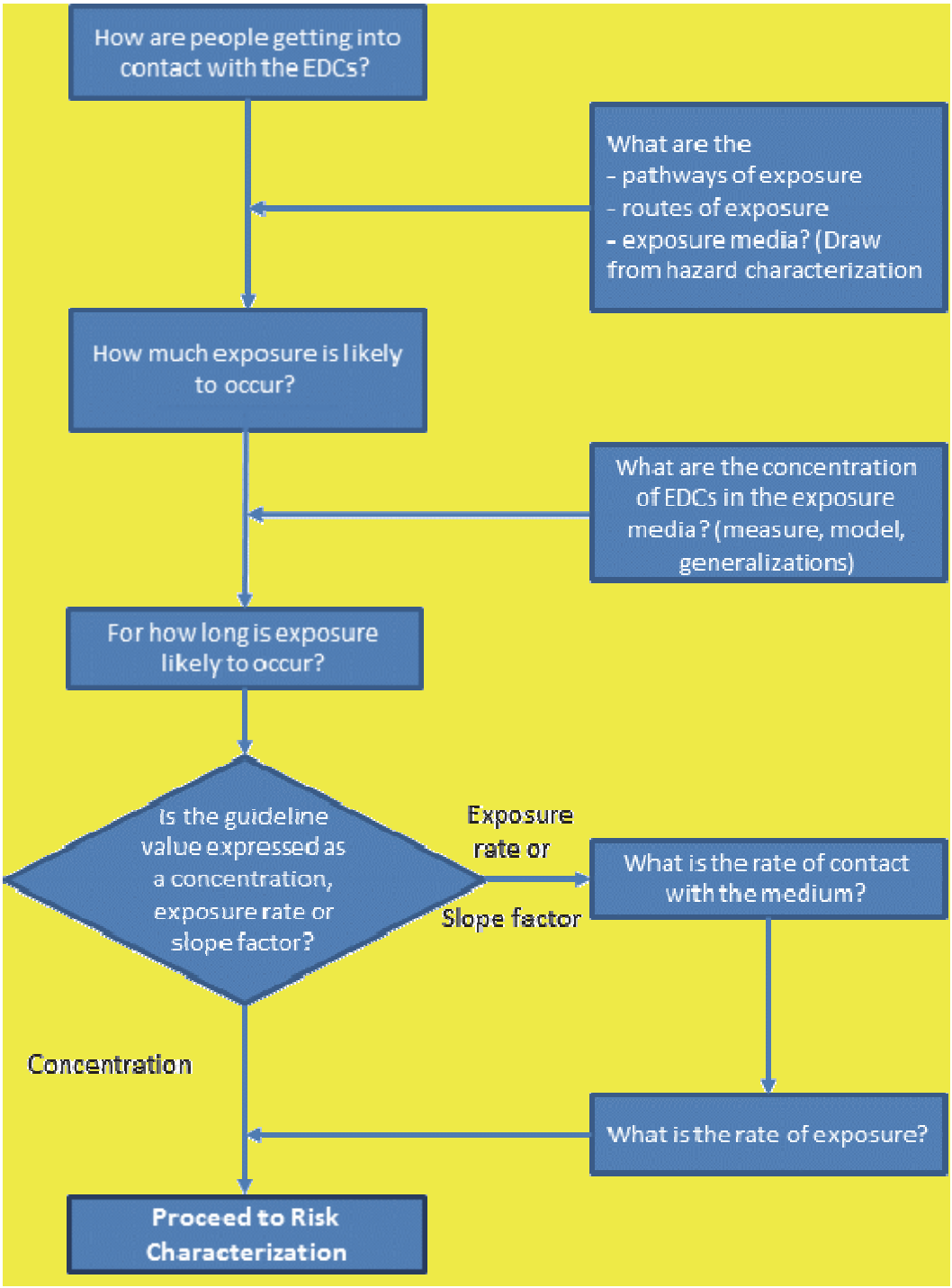


Figure 2: Generic road map for exposure assessment [68].

Risk characterization

- *How does the estimated exposure compare with the guidance or guideline values*?

Risk characterization consists of comparing an estimate of chemical exposure with a guidance or guideline value. For non-carcinogenic effects, this would be the reference dose (Rfd), given by the WHO and US EPA, and for carcinogenic health effects a potency or slope factor is used to calculate a quantitative cancer risk.

Does the measured endocrine activity exceed the recommended trigger value for drinking water? Determining the endocrine activity through bio-assay serves as a screening for possible endocrine activity. If the trigger value is exceeded, more detailed analysis in specific chemical compounds can be done (and follow the procedures as recommended in the previous section).

These chemicals are shortlisted by their likelihood of occurrence in a given resource (for example pesticide evaluation close to agricultural practices) and their priority listing given by the USEPA. A summarization of the risk characterization is made in Figure 3.

The assessor is now armed with the hazard quotients and cancer risk as well as the extent to which the trigger value is exceeded. There is currently not enough information or data on linking hazard quotient calculation to observed endocrine activity and only in a few studies have endocrine effects been considered when developing a RfD.

As recommended by the US EPA, a prioritization of chemicals determined in the water sample is recommended (See section on prioritization of EDCs). Many chemicals, many of which are metals and metal complexes have not yet been considered by the US EPA for prioritization, due to a lack of supporting data. Metals with high calculated hazard quotients should enjoy priority for further investigation into their possible contribution to the observed endocrine activity in a given water sample.

Figure 3: A generic map for risk characterisation [68].

Summary

- Once the priority chemicals have been identified the possible environmental or anthropogenic sources should be investigated and the sites were they were detected should be surveyed for land and water use impacts.

- The toxicological studies of the priority pollutants should be scrutinised and where information regarding sentinel specie exposures is available, it is highly recommended to include it into the health risk assessment.

- New methods for evaluating endocrine behaviour and carcinogenic effects are continually being developed and advanced, and assessors will need to keep abreast of all these developments.

- If funds allow, monitoring of the priority pollutants should continue to determine seasonal impacts and speciation of pollutants would provide information of toxic fractions of given elements.

4. CASE STUDIES

4.1 Introduction

One of the common objectives of the Volumes of this Manual is to highlight research needs and to thus also stimulate further EDC research. In order to achieve this it was necessary to ascertain the current capacity and capabilities in the context of EDCs and water quality with reference to monitoring and assessment applicable in the South African context.

This was performed by conducting several case studies as part of Deliverable 6 for the WRC Project K5/1915 in order to test the methods presented in the Volumes. The full text for the case studies is comprehensive and contains the application of many of the processes detailed in other Volumes of the Manual. These include the sampling methodology, analytical procedures, bioassays and assessments incorporating histopathological investigations.

The extent to which EDCs were monitored and assessed by multiple environmental companies in accordance with the current legislation as stipulated by various authorizations and licences applicable to the Kusile Power Station (KPS) and related activities was also evaluated.

A sentinel monitoring and assessment methodology was also investigated as a supporting tool for EDC research and detailed for multiple case study sites. This methodology is also being employed in another WRC project involving EDCs (WRC Project K5/1956) and the reader is referred to the actual Deliverable and other projects for further detail. This section provides a brief overview of the case studies conducted and lessons learnt from the process.

In selecting the case study sites an additional key consideration was that whilst targeted EDC research involving public health is recommended, due to the high costs, time-frame required for ethical approval and community involvement, a human-health risk assessment would not be performed, but the case studies would focus on addressing the key requirements for assessing the impacts on animal health and related norms.

The use of animal models for assessing EDCs is arguably a preferable route in terms of EDC research as many of the key effects attributed to EDCs are reported in the scientific literature in animal models. The use of animal models as sentinels for monitoring within the context of public health is also generally accepted.

The case study sites subsequently addressed two species, namely broilers (poultry) and pigs. These are animals used in intensive commercial production systems with several key benefits, primarily a reduction in variation. This contributes to confidence in attributing potential EDC effects to the exposures described to EDCs.

In many cases these systems are breed-specific and due to the commercial application multiple sites across South Africa will employ standard management systems, from health care, biosecurity, nutrition to environmental variables. This allows for meaningful extrapolation across differing EDC exposure sites.

These system types are thus specific and provide detail that not serves as an input for exposure information but critically includes ingestion data. Exposure assessment is thus an actual measurement based on a high number of replicates and repetitions which allow for correction of environmental variables and influences (e.g. season and climatic conditions) accurately measured and not merely estimated.

The key benefit is that aspects of source, pathway and exposure, may be isolated and controlled and accurately sampled and assessed. This is seldom, if ever, the case in human exposure studies. Intensive systems are also characterized by high biosecurity measures and herd health disease controls allowing for quick comparisons between sample groups and sites in terms of normality, and as such allow for significant uncertainty reduction. The significant reduction in confounding factors and exposure certainty increases the confidence in linking observed EDC effects to exposure to EDCs.

Since the EDC Manual has water as the central point of departure it is furthermore understandable that the approach is linked centrally to water and water quality data. The central focus is on initial baseline condition description based on which a Priority List of EDC Chemicals is determined and used to formulate the required steps to assess animal health.

Baseline description is a critical step and it should be standard procedure to assess the exposure to all significant hazards in order to determine context under which any suspected adverse effect (e.g. an EDC effect) may be considered. This recognition of adverse effects following exposure to routinely used chemicals in animal production is widely reported in the scientific literature and is too extensive to review here.

Fundamentally similar is the appreciation of human exposure to other EDCs in routine use of various pharmaceuticals and exposures to a variety of potentially hazardous substances outside of EDCs applicable to recognized domestic uses of water.

Failure to take note of these background factors will render any clinical or diagnostic statements made with respect to EDCs and related effects open to criticism regarding alternative routes of toxicity, adverse effects and/or conditions predisposing disorder, disease or disruption.

The case studies selected were divided into two sets.

- **Set A:**
  - Addressed three different sites utilising poultry as the model to investigate potential EDC hazards
- **Set B:**
  - Kusile Power Station (KPS) and Pig Genetics Facility (PGF) using a porcine model to investigate potential EDC hazards.

The general objective of these case study sites was not to provide an exhaustive review of an ideal EDC investigation from start to finish, but rather to place emphasis on the importance of including the relevant aspects necessary for meaningful EDC investigations.

Common errors noted regarding the sampling and analysis with reference to existing monitoring activities are also presented. In so-doing an outcome was to guide other EDC studies not only by highlighting the various steps required, but to also draw attention to routine errors that often preclude meaningful EDC assessments.

The inclusion of the various EDC considerations throughout the case studies is viewed as a prerequisite to allow for meaningful EDC research to be conducted which in turn should allow for investigations to lead to regulator / health authorities making decisions based on defensible scientific data.

The contribution of EDCs is also viewed as a fundamental requirement for not only contextualising EDC effects or bioassay results within the description of baseline conditions, but also for meaningful monitoring programmes to be designed. This combination of factors may thus provide inputs for catchment management.

4.2 Case Study Focus

As the organic EDC component has already received attention via several WRC reports and is also addressed in a WRC Project K5/1956 (due for completion 2015), the focus on the case study sites were on inorganic EDCs. Similarly, as the oestrogenic and anti-androgenic EDC effects have received attention via several WRC reports, in addition to employing the bioassays detailed in the Toolkit androgenic and thyroid disruption were also investigated. Links to these WRC reports are provided in the reference list.

As the Volumes in the Manual are intended to provide some form of management guidance regarding EDCs meaningful inputs towards this final phase are required. These may be conducted to a varying degree of accuracy from initial generic assessments, the use of screening tools, and finally to more site-specific investigations. Typically there is a prioritisation required for the extent to which a specific

site or catchment should be investigated for EDCs. Each additional step adding to the investigation tends to encompass more specialised tasks and it thus reasonable to expect a step-wise progression with outcomes from preceding steps serving to motivate and provide direction for the next stage.

A major challenge with regards to EDC investigation is that in order to interpret hazards and risks several uncertainties need to be described. The general methodology applied is thus a sequenced approach involving the source, pathway and receptor and may involve the following key components:

- Description of baseline conditions to allow for the relevance of exposure to be determined.
- EDC assessments that include and thus recognize the components that may be naturally occurring as well as due to hazardous contaminants or pollutants.
- Concurrent assessments in relevant exposure media (air, water, and feed) and clinically relevant tissues (blood, liver, kidney and thyroid) are required to perform a differential diagnosis which is a significant tool in describing EDC effects.

These components allow for meaningful risk factor identification which in turn allows management to make decisions on mitigation measures. A combined approach to the assessment and monitoring is required with each of the bioassay screening, organic and inorganic chemical approaches relevant, at times for differing reasons. The large numbers and ultra-trace concentrations of relevance to EDC effects from organic compounds in environmental matrices is problematic from both an analytical methodology and high cost of analytical determination perspective. Although less challenging in this regard the inorganic constituents present similar problems as awareness increases regarding the lists of candidate chemicals and exposure concentrations of relevance to endocrine disruption.

Both organic and inorganic constituents share the concern that they may be presented to the affected user (receptor) in a matrix, or mixture. Exposure to mixtures may also be concurrent or sequential and varied concentrations. This can result in a wide variety of interactions and outcomes that range from supra-additive to infra-additive. This significantly complicates the assessment of hazards and risks.

Consequently, biological methods are addressed in Volume 3 as screening tools. This approach is of great value when the specific chemical composition of the exposure is unknown. There are, however, limitations to the use of bioassays to predict outcome under exposure conditions as they remain screening tools that should be used to decide on further steps in hazard and risk assessment processes. More suitable and reliable bioassays are continually being developed and their role in EDC research continues to grow.

The US Endocrine Society released a Scientific Statement in 2009 [8] in which a comprehensive review was published regarding EDCs. In the Position Statement released [69] it was observed that:

- There is no comprehensive coordinated approach to regulating EDCs in the US.

- The review that focuses on low-dose exposure to EDCs on endocrine systems, clearly elaborates a strong basis for concern about EDC health risks.

- Policies that fail to adequately consider these low-dose effects…could lead to regulatory decisions that inappropriately define safe levels for some EDCs.

- For many chemicals in use today, no data exist on their EDC activity.

- EDC policy relevant to public health must be based on analysis of both low- and high-dose actions, as well as both short- and long-term exposures and simultaneous exposure to multiple common EDCs.

- Timing of exposure is critical considering the increased sensitivity and vulnerability of foetuses and infants.

In a Global Water Research Coalition Review on bioanalytical tools to evaluate hormonal activity in environmental waters [10], similar conclusions were arrived at. It was noted that whilst much research has been conducted into the oestrogenic aspects it is clear that other endocrine endpoints can be disrupted by exposure to EDCs. It was also observed that most bioassays have been applied "patchily" to measure endocrine activity in wastewater, and there is little information about non-oestrogenic activity in other waters, including drinking water, surface and groundwater. This case study section addresses some of these aspects by investigating surface and groundwater used for drinking purposes with a combination of bioassays and other tools for thyroid investigation employed.

Despite these statements being valid it cannot reasonably be expected for routine assessment and monitoring to account for all the possibilities and constituents relevant. The step-wise source, pathway and receptor approach is thus viewed in context of the reality of budget and time constraints and applied in order to provide sufficient motivation to include, or exclude, certain testing procedures.

It should be appreciated that the design of both assessment investigations and monitoring programmes may be sufficient from one perspective, for example decision-making relevant to catchment management, but not necessarily so from a different perspective, for example scientific research into EDC cause and effect relationships.

It follows that the general objective of the case study sites was not to provide an exhaustive review of an ideal EDC investigation from start to finish, but rather to place emphasis on the importance of including the relevant aspects necessary for meaningful EDC investigations. Common errors noted regarding the sampling and analysis with reference to existing monitoring activities are also

presented. In so-doing an outcome was to guide other EDC studies not only by highlighting the various steps required, but to also draw attention to routine errors that often preclude meaningful EDC assessments.

The inclusion of the various EDC considerations throughout the case studies is viewed as a prerequisite to allow for meaningful EDC research to be conducted which in turn should allow for investigations to lead to regulator / health authorities making decisions based on defensible scientific data.

The contribution of EDCs is also viewed as a fundamental requirement for not only contextualising EDC effects or bioassay results within the description of baseline conditions, but also for meaningful monitoring programmes to be designed. This combination of factors may thus provide inputs for catchment management.

4.3 Generic Approach

This section provides a brief overview of the steps involved in the water quality investigations employed, following which more specific detail on the case study sites is presented. The steps followed when conducting water quality investigations entail sampling, testing (performing analytical procedures), and an assessment. These are determined to a large extent by the objectives of the investigation, which are in turn usually prompted by the following considerations:

- a perceived water quality problem
- a requirement for additional volume and thus source testing
- due diligence prior to acquiring property/water use sector
- research objective driven

Increasingly water quality testing is being conducted as part of environmental impact assessments, general authorisations for controlled water use activities and water use licence applications, or as part of good management practice. Although other sample media types were included and described in the case studies, the water component is highlighted here.

4.3.1 Water Sampling

The key steps involved in the water quality investigations are highlighted in Figure 4.

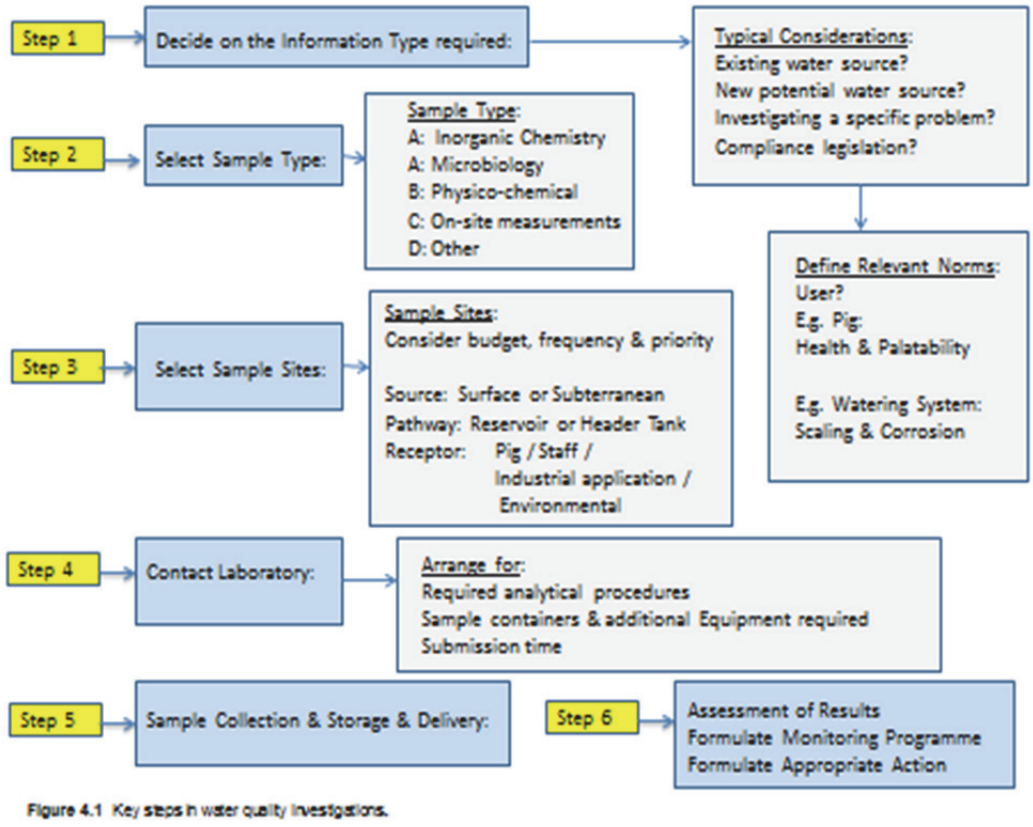


Figure 4.1 Key steps in water quality investigations.

4.3.2 Selecting Sample Sites

Although the sample site selection may be best determined by evaluation of the site prior to sampling this is not always practically feasible or clinically indicated. Sample site selection may follow a process of elimination after evaluating initial results leading to priority sample sites being selected. The following considerations apply:

- A sources, pathways and receptor approach
- A priority for exposure assessment (point of use)

Should uncertainty exist regarding the relevant pathways for an exposed receptor then the focus may be on different pathway routes and this may extend to different sample media. However, despite the variability of the sources and pathways involved it may be considered critical to obtain a sample representative of exposure. This is in order to be able to formulate statements on hazards and risks for exposed receptor types in the user groups potentially affected.

For EDC health-related assessments this step is critical without which the investigation will focus more on pollution or potentially hazardous substance source and pathway aspects and be unable to provide a more accurate statement of EDC exposure hazards and risks.

An additional consideration supporting the need for point of use (receptor) sampling is that some EDCs may form or arise from processes in the water distribution system. For example, bromide may be present in the water source but disinfection with chlorine prior to presentation to the receptor (user) can result in the formation of disinfection by-products that may be endocrine disruptive or recognised to result in the formation of toxic or carcinogenic by-products, such as bromate.

It should also be noted that in the case of nitrate-related endocrine effects, the concentration can increase significantly after boiling or exposure to high temperatures, accordingly point of use concentrations are often greater than source observations. The addition to point of use quality by contamination of source or stored water may also be significant, with some fittings and fixtures and cleaning chemicals capable of leading to increased exposure concentrations. For some constituents numerous considerations may apply, for example, evaporative effects, Eh and pH conditions. The issue requiring consideration is that the concentrations and exposures may vary significantly between source, storage to the point of use and need to therefore be adequately described. Thus, failure to sample the point of use may thus lead to a significant underestimation of the point of use exposure concentrations.

Finally, as a general consideration for EDCs the use of a reference site warrants consideration. In many cases insufficient reference values may be available for the elements of research interest and the comparison between the sites investigated that may be suspected of having EDCs with a site that can potentially serve as a control site is advantageous. This is also true for the histopathological evaluation of selected tissue types and evidenced by the results presented later.

Notes:

- The objective of the sampling will determine to a large extent the sample site/s selected. This may range from a specific EDC effect investigation regarding an identified receptor to monitoring for legislative compliance.
- The general approach should consider the sources, pathways and receptors relevant to EDCs.
- A stepwise approach is recommended which may allow for the collection of samples that may either be discarded or utilised depending on the outcome of a series of analytical results.
- Sampling must yield a sample suitable for the testing of the relevant water quality constituents (WQC) being investigated.

4.3.3 Selecting Sample Tests

Water quality is a term used to describe the chemical, physical and microbiological properties thereof. These properties may be used to assess the fitness for the intended use. Fitness for use descriptions

linked to a WQC and supporting information is used to provide some guidance on the types of effects that can be expected when using the water for a specific purpose. Further detail in this regard may be obtained from the 1996 South African Water Quality Guidelines (DWAF, 1996, Volumes 1 to 8) [2].

A step-wise approach may be followed with an initial screen after which targeted sampling may be conducted. An example of this would be in the case of pesticides, where an initial screen is conducted prior to testing for specific compounds or ingredients. Microbiological indicator organisms are another example where due to cost considerations water is tested for indicator organisms as opposed to the actual pathogens. If the indicator organism results suggest that the water may well be a potentially significant source of water-borne pathogens then further targeted testing for specific pathogens may be indicated.

Laboratories may have their own specific sample collection procedures and it is thus advisable to first check regarding the preferred collection methods. This may extend to the provision of different types of sample containers that may be prepared by the laboratory. It is also advisable to arrange sample delivery with the laboratory prior to setting a collection date. This is to accommodate the possibility that some laboratory apparatuses may be fully utilized during specific periods when high volumes of samples are submitted routinely and may thus not be able to process the samples submitted within the requested time-frame. Specific submission times may be required, for example, with microbiological samples. Should monitoring be planned then an agreement regarding the sample delivery schedule should also be reached. The following sample tests may be considered:

Type A: Generic Approach

- Inorganic Chemistry
- Microbiological Indicator Organisms

Type B: Source pollution or discharge compliance

- Physico-chemical properties

Type C: On-site tests

- Disinfection residuals: Typically for free chlorine
- Portable instrument / test strip tests: e.g. pH & nitrate & Electrical Conductivity

Type D: Organic compounds

- Organic Water Quality Constituents: Water Research Commission Report: 1774/1/08 (Burger, AEC & Nel, A, 2008/09/01) from www.wrc.org.za.

Type E: Water for application in EDC Bioassays

- Oestrogenic Assays: Water Research Commission Report: 1816/1/10 (De Jager, Aneck-Hahn, Barnhoorn, Bornman, Pieters, van Wyk and van Zijl) from www.wrc.org.za.
- Androgenic and Anti-androgenic Assays: Water Research Commission Project: K5/1956/Deliverable 6: Chapter 3. R Pieters. From www.wrc.org.za

A key advantage of performing Type E tests is the ability to screen for the responses to a "mixture" of EDCs potentially present. The result does not necessarily identify the causative EDCs but the procedure does take into account the fact that the simultaneous presence of multiple different types of EDCs may collectively produce an endocrine disruptive effect, whilst individually they may not.

The following tables highlight the information required for these test types. It is important to check that the laboratory to be used will in fact provide this detail. Additionally, the detection limits need to be sufficiently sensitive. It is strongly advised not to conduct partial testing as this often precludes a meaningful assessment from being performed and results in questions being posed regarding uncertainty in the data gaps that subsequently arise. Since many EDCs and endocrine disrupting effects are contentious it is best to avoid having significant data gaps. Thus, failing to describe the chemistry adequately can prevent a differential diagnosis from being formulated as interactions are insufficiently described.

The macro element determination will typically be conducted on a full quantitative basis with Anions by Ion Chromatography and Cations by ICP-OES.

It is strongly recommended to use ICP-MS (Inductively coupled plasma- mass spectrophotometry) for the trace elements.

Two general types are available. The first is utilised by the Institute for Soil, Climate and Water (ISCW) at the Agricultural Research Council (ARC) which is a semi-quantitative scan, whist the second is a full quantitative ICP-MS procedure employed by the Pelindaba Analytical Laboratories

(PAL) at the South African Nuclear Energy Corporation (NECSA). Cost is the major consideration with the ISCW scan sufficient for most investigations and monitoring programmes, specifically if multiple sample media types are being investigated (soil, biological). The PAL process is recommended for compliance testing and in cases where legal action is being considered. The ISCW is part of the AGRILASA scheme (inter-laboratory testing) and PAL is a SANAS accredited laboratory.

Further references may be obtained from the following sources:

- Standard Methods for the Examination of Water and Wastewater (1985). 16th Edition. American Public Health Association. Washington DC 20005. ISBN: 0-87553-131-8
- SANS 5221:2007 (Edit 4.3): Microbiological quality of water: General Test Methods.
- F.M. 5.4 W-B: Total coliforms and *Escherichia coli* in water – Defined Substrate Technology (Colilert) Method.
- SANS 241-1:2011 Edition 1. Bibliography. Standards. Pages 12-14. Annual book of ASTM standards and SANS Standard Test methods.
- Sampling and Methods for the analysis of Inorganic Endocrine Disrupting Chemicals: Water Research Commission Report K8/999.

<table>
<tr><th colspan="2">Type A: Inorganic</th></tr>
<tr><th colspan="2">Inorganic Macro Elements
Method: IC</th></tr>
<tr><td>Anions:
Fluoride
Nitrite; Nitrate
Chloride; Sulphate
Carbonate; Phosphate; Bicarbonate</td><td>Cations:
Sodium; Potassium
Calcium; Magnesium; Boron</td></tr>
<tr><td colspan="2">Additional parameters calculated or included:
Total Dissolved Solids & Electrical Conductivity & pH (actual and saturation) & SAR
Alkalinity & Sodium Bicarbonate & Sodium Carbonate
Permanent Hardness &Temporary Hardness</td></tr>
<tr><th colspan="2">Inorganic Trace Elements:
Method: ICP-MS</th></tr>
<tr><td colspan="2">Aluminium Antimony Arsenic
Barium Beryllium Boron Bromide
Cadmium Caesium Chromium Cobalt Copper
Iodine* Iron^ Lead Lithium Lanthanum
Manganese Mercury Molybdenum Nickel
Selenium Silver Strontium
Tellurium Tin Titanium Thallium Tungsten
Uranium Vanadium Zinc</td></tr>
</table>

Note:

The sensitivity required for guideline application is:

0.001- 0.0001 mg/L

Some guidelines are below 0.01 mg/L (e.g. cadmium at 0.003 mg/L) but most are in the 0.01 to 0.1 mg/L range and the trend is towards lower limits being applied. The ICP-MS approach utilized should enable accuracy for the sub-0.001 mg/L range and thus be of benefit for future lowered target water quality guideline ranges.

**Iodine* = typically removed due to interferences. ^ Iron = often performed separately due to interferences.

Type A: Microbiological
Microbiological Indicator Organisms: **Total Bacteria Count** (Counts/mL); **Total Coliform Count** (Counts/100 mL); ***E. coli*** (Counts/100 mL)
Note: The Total Bacteria Count is similar to a Total Plate Count or a Heterotrophic plate count. Sometimes Faecal Coliforms will be reported and in the absence of *E. coli* may be used instead. The choice between the two is dependent on a variety of study objectives.

Type B: Physico-chemical
Chemical Oxygen Demand reported as O_2 mg/L **Suspended Solids** at 105^0C reported as mg/L **Surfactants** reported as mg/L **Free and Saline Ammonia** reported as N in mg/L
Note: Effluent discharge may impact on receiving water quality with EDCs also noted to occur in effluent. Surfactants are similar to soaps, oils and greases and conducted when such pollutants are suspected.

Type E: Bioassays
Collection of water samples (and sediment) for oestrogenic,androgenic and anti-androgenic activity determined by**:** **Yeast Oestrogen Assay** **T47D-KBLUC Oestrogen Assay** **MDA-kb2 Androgen Assay**
Note: The collection procedure is very specific and storage procedures also vary for different types of water samples. Refer to Water Research Commission Report No: 1816/1/10 page 4 for specific detail regarding the oestrogenic assays. Refer to Water Research Commission Project: K5/1956/Deliverable 6 Chapter 3 for the androgenic assays.

Detail concerning the pre-sampling preparations, equipment required, collection procedure accessories, data capturing guides, and laboratory methods are presented in the Deliverable 6 for WRC K5/1915 and not repeated here.

Note:

- The use of ICP-MS for the list of inorganic WQCs presented in this section is strongly recommended in order to assess the hazards and risks posed by exposure to EDCs.

- An investigation regarding EDCs in water should be accompanied by an adequate description of the relevant chemistry in order to describe baseline conditions. Recognition for naturally occurring and anthropogenic inorganic constituents is needed not only for their direct EDC effects but also for interactions with recognized EDCs.

- Failure to address this topic may compromise the interpretation of other EDC screening methods and may introduce additional uncertainty in the assessment of effects following exposure to EDCs.

- As new EDC research is continually made available more inorganic constituents are considered relevant, usually concurrently with the recognition of endocrine effects at lower concentrations. The application of inorganic water quality data collected and monitoring programmes designed should consider these aspects involving constituent selection and detection limits.

4.4 Case Study Site Selection Considerations

The case studies were divided into two sets. One investigated the use of poultry as a sentinel model for investigating EDC effects, whilst the other utilised a single site with extensive existing monitoring programmes to evaluate the ability of current applications of relevant environmental and water-related legislation, authorisations and licences in terms of the quality of data produced and EDC relevance, with a porcine model used for sentinel purposes.

A key consideration in the selection process was that whilst targeted EDC research involving public health is recommended, due to the high costs, time-frame required for ethical approval and community involvement, a human-health risk assessment would often not be performed. Consequently, the case studies focussed on key requirements for assessing the impacts on animal health and related norms. The procedure employed would thus serve to assist future studies that could use the animal assessment outcomes for EDC exposures as motivation for required human-health risk assessments or prioritization of EDC endpoint investigations.

The use of animal models for assessing EDCs is arguably a preferable route in terms of EDC research as many of the key effects attributed to EDCs are reported in the scientific literature in animal models. The use of animal models as sentinels for monitoring within the context of public health is also generally accepted. Fundamentally, the primary benefit is from a reduction in variation. This contributes to confidence in attributing potential EDC effects to the exposures described to EDCs.

In many cases these systems are breed-specific and due to the commercial application multiple sites across South Africa will employ standard management systems, from health care, biosecurity, nutrition to environmental variables. This allows for meaningful extrapolation across differing EDC exposure sites.

These system types are thus specific and provide detail that not only serves as an input for exposure information, but also includes ingestion data. Exposure assessment is thus an actual measurement based on a high number of replicates and repetitions which allow for correction of environmental variables and influences (e.g. seasonal effects) accurately measured and not merely an estimate thereof.

The key benefit is that aspects of source, pathway and exposure, may be isolated and controlled and accurately sampled and assessed.

This is seldom, if ever, the case in human exposure studies. Intensive systems are also characterized by high biosecurity measures and herd health disease controls allowing for quick comparisons between sample groups and sites in terms of normality, and as such allow for significant uncertainty reduction. The significant reduction in confounding factors and exposure certainty increases the confidence in linking observed EDC effects to exposure to EDCs.

Since the EDC Manual has water as the central point of departure it is furthermore understandable that the approach is linked centrally to water and water quality data.

4.5 Methodology

4.5.1 Approach

As noted earlier, site detail is not presented here and the reader is referred to Deliverable 6 for WRC K5/1915 for reference purposes. The first step conducted was an initial site-specific description to assess the background exposure to significant hazards in order to determine baseline conditions under which any suspected adverse effect (e.g. an EDC effect) may be considered. This recognition of adverse effects following exposure to routinely used chemicals in animal production is fundamentally similar to the appreciation of human exposure to other EDCs in routine use of various pharmaceuticals and exposures to a variety of potentially hazardous substances outside of EDCs applicable to recognized domestic uses of water.

Failure to take note of these background factors may render any clinical or diagnostic statements made with respect to EDCs and related effects open to criticism regarding alternative routes of toxicity, adverse effects and/or conditions predisposing disorder, disease or disruption.

A combination of standards and guidelines may be consulted for detailed descriptions of sampling activities from design, collection, and storage to handling. It is generally recommended that when conducting any EDC research the relevant laboratory should be consulted for detail regarding method reference source and the specific analytical standard methods as these may vary and be adapted to specific brands of analytical apparatuses. Additional detail applicable to the sampling protocol in terms of site and sample site selection is provided in the volumes on Sampling (Volume 2), Toolkit for Bioassays (Volume 3), Assessment and Monitoring (Volume 4) and in the WRC Report K8/999.

The case studies selected were divided into two sets.

- **Set A: Sentinel Investigation**
  - Addressed three different sites utilising poultry as a model to investigate EDC hazards
    - **Site 1** = Surface Water: Komatipoort Area, Mpumalanga Province
    - **Site 2** = Groundwater: Bonjanala Region, North West Province
    - **Site 3** = Municipal water: Springs Area, Gauteng Province

- **Set B: Existing Monitoring and Assessment Investigation**
  - Kusile Power Station using a porcine model to investigate EDC hazards
    - Using a single site with extensive existing monitoring programmes to evaluate how current applications of environmental and water-related legislation, authorisations and licences produced data relevant to EDC effects.

The objective of these case study sites was not to provide an exhaustive review of an ideal EDC investigation from start to finish, but rather to place emphasis on the importance of including the relevant aspects necessary for meaningful EDC investigations. Common errors noted regarding the sampling and analysis with reference to existing monitoring activities are also presented. In so-doing an outcome of this deliverable is to guide other EDC studies not only by highlighting the various steps required, but to also draw attention to routine errors that often preclude meaningful EDC assessments.

The inclusion of the various EDC considerations throughout the case studies is viewed as a prerequisite to allow for meaningful EDC research to be conducted which in turn should allow for investigations to lead to regulator / health authorities making decisions based on defensible scientific data.

The contribution of EDCs is also viewed as a fundamental requirement for not only contextualising EDC effects or bioassay results within the description of baseline conditions, but also for meaningful monitoring programmes to be designed. This combination of factors may thus provide inputs for catchment management.

4.5.2 Brief Overview of Assessment Methods

Several different assessment methods are applicable and relevant to the sample type and user (receptor) potentially affected. Only the key issues are noted in this section with further detail in Deliverable 6 for K5/1915.

4.5.2.1 Water Quality

Water Quality as a Function of the Intended Use

The South African Water Quality Guidelines (DWAF, 1996) [2] employ a “fitness for use” approach in order to determine or describe water quality. Water quality is defined in terms of the type of intended use and not simply or generically as poor or good or “potable”. As an example, water that may be fit for humans to drink may not be suitable for use for irrigation purposes due to long-term soil effects. The one emphasis may thus be on health whilst the other on environmental/production-related issues.

Water quality constituents (WQC) refer to properties of water and/or substances suspended or dissolved in the water. A fluoride concentration in water of 2 mg/L simply provides the analytical evidence describing the constituent without offering any statement on whether this represents a desirable concentration or not. It does not offer any guidance on possible effects based on drinking water exposure or the use of such water for irrigation purposes. For this some narrative is required linking the concentration in terms of the effects that may occur for the specific use. Fitness for use thus uses constituents and supporting descriptions to provide some guidance on the types of effects that can be expected when using the water for a specific purpose. These types of effects may be considered as affecting a wide range of different aspects of the same use.

Thus drinking water provided for pigs may also affect other aspects of the production system that may have effects not directly linked to pig health. For example, the water may impact on the replacement of water distribution system fittings and fixtures but affecting scaling and corrosion attributes. These different types of effect categories are referred to as "norms", and each water use type may require different norms to be considered in order to arrive at a statement on the "fitness for use".

The combination of the constituent concentration ranges and types of effects are used to develop water quality guidelines which are then used to judge the "fitness for use" for the specific purpose. The South African Water Quality Guidelines (DWAF, 1996) presents separate volumes for the recognized uses, namely:

Volume 1: Domestic Water Use
Volume 2: Recreational Water Use
Volume 3: Industrial Water Use
Volume 4: Agricultural Water Use: Irrigation
Volume 5: Agricultural Water Use: Livestock Watering
Volume 6: Agricultural Water Use: Aquaculture
Volume 7: Aquatic Ecosystems

The results are reported using the following categories:

Concentrations recorded which exceed the Department of Water Affairs and Forestry (DWAF, 1996) guideline for the category C, or the internationally recommended upper guideline limit, are reported as potentially hazardous chemical constituents (PHCC). Constituents that recorded values within 10% of a guideline limit are reported as constituents of concern (COC). Definitions of these terms appear below.

WQC:	Water quality constituent, e.g. Arsenic.
PHCC:	Indicates that exposure to the WQC in question is likely to result in adverse effects.
COC:	Indicates that the WQC in question could conceivably become a PHCC due to concentration variations, such as seasonal fluctuation in the water source or evaporative effects, and should therefore be monitored.

Additional acronyms used include:

AV	antagonistic variable (Underwood & Suttle, 1999)
Cat C	risk of adverse effects in sensitive user groups
Cat D	risk of adverse chronic effects in all users
Cat E	risk of adverse chronic and acute effects in all users (Quality of Domestic Water Supplies, 1998)

MRL maximum recommended limit (WHO, 1996)
TWQR target water quality range (DWAF, 1996)

4.5.2.2 Bioassays

Due to the limitations of the yeast screen oestrogenic assay the T47D-KBLUC reporter gene assay was also included in combination with the YES assay. This procedure is recommended in the reference document (WRC Report No: 1816/1/10).

Most of the assessments regarding oestrogenicity involve a colour change which is detected and reported on. For the YES assay colour development is read after a 3 day incubation period using a plate reader (Titertek Multiskan MCC/340) and a series of equations are used that incorporate the detection limit of the assay with oestradiol equivalents calculated for those samples with 3 or more points above the detection limit.

In the case of the T47D-KBLUC assay luciferase activity is determined using a luminometer with the results used to calculate oestradiol equivalents. These may in turn be used for risk assessment statements.

In the case of the androgenic bioassay samples are evaluated for activation with luminescence determined in a plate reader (Berthold multimode micro plate reader, model-LB941). The sample responses are reported as relative responses units that are expressed as a percentage relative to the maximum response obtained.

It is important to note that the bioassay-derived responses are derived by using a regression equation for the appropriate standard curve with calculation using a bioassay response equal in magnitude to that induced by the sample in question.

Further detail regarding the techniques, derivation and comparison methods for the MDA-kb2 androgenic bioassay, including androgenic activation, inhibition and cytotoxicity, may be obtained from the full bioassay report which is submitted with this Deliverable as an attachment.

4.5.2.3 Histopathology

The processed sections were examined by a specialist pathologist using standard techniques. As the samples were obtained from commercial broiler breeds for Set A sites this was conducted by poultry pathologist specializing in commercial poultry production breeds.

4.5.2.4 Tissue Values

The processed specimen samples were pooled as observations per site and compared with reference values for broiler and poultry tissue values for inorganic constituents. Although this may be expanded for further assessments of deficiencies, adequacies and excesses, the objective of this investigation

was to focus on differences between sites that may be reflected by histopathological observations and supported by blood hormone values. Median values were used for comparisons as opposed to mean values due to the stochastic variability inherent in tissue element values.

4.6 Case Study Set A: Poultry Production Sites

4.6.1 Background

The primary motivation for the use of poultry as sentinels for EDC effects is that both the source and pathway are defined. Consequently the receptor evaluated offers significant observations in terms of replicates and repetitions. As an example the one site used places ca. 100 000 broilers per 35 day growth cycle evenly distributed across 6 identical houses. This placement is repeated after a 10 to 14 day clean-out phase with around 7.5 cycles completed per year, allowing for a similar approach to be used typical of an experimental design with repetitions and replicates, enabling seasonal variations to be corrected for. The placement may also be replicated across multiple different sites across South Africa within commercial companies, allowing for meaningful comparison of different environmental conditions.

The use of poultry as sentinels for public health is used in the USA for monitoring of mosquito-borne diseases and offers possible links regarding other WRC and health-related investigations. A key factor in the rural communal system application is that broilers produced are typically consumed entirely, often having been offered the same EDCs via the drinking water that are used as a common water source for food preparation, irrigation of household crops and the various domestic water uses applicable to the community involved. This has been shown to significantly affect both tissue values in terms of maximum acceptable concentrations due to accumulation effects and also to fitness for use within the community due to the lack of dietary dilution [70].

Hence, the use of the poultry products as a significant component of rural community diets allow for tissue assessments for EDCs to be of value for both the investigation of EDC effects in the poultry and exposure assessments for public health.

In order to remove any significant breed or strain variability each of the three sites utilises the same breed and strain, namely the Ross 308. This is a commercial broiler breed widely used by the major broiler growers in South Africa and world-wide. The production sites are thus all broiler production sites that may obtain genetic material (chicks to be placed) from the same breeder farm and flocks and even the same hatchery. This is a critical reduction in potential variations with regards to the expression of EDC effects.

Three broiler sites were chosen following consultations with specialist poultry veterinarians and a poultry pathologist, with the same breed used. Consequently, the management applications in terms of vaccinations, environmental control targets and nutritional programme followed are usually part of

standard operating procedures within commercial companies but are furthermore generally similar even between the large commercial producers.

Additional benefits relate to the low cost of sample acquisition and sample mass required. All the key endocrine-related tissues are routinely obtained at post-mortems and the methodology employed may also be easily acquired. Recordings of daily feed intakes, water intakes, mortalities, systematic lesion scoring and serology are also often conducted as a routine procedure and assist greatly with identifying normality within the observation sample cohorts selected.

The variation between source and point of use has to be taken into account as many sites practice disinfection by chlorination with disinfection by-products being formed that may be potential EDCs.

The benefit of utilizing a team of veterinarians (pathologist and nutritional pathologist) allows for the screening of confounding diseases in the effects assessment to exposure to EDCs.

In summary, the production system context incorporated for the case study set A also represents a viable methodology for achieving one of the other stated aims of the EDC Manual, namely to stimulate and guide research at other institutions throughout the country. This is due to the fact that the major commercial role-players have growing facilities across the country in order to access local market chains. Different EDC exposure effects may thus be also investigated across diverse environmental conditions, from rainfall, temperature to altitude.

4.6.2 EDC Exposure Sites

Three different sites were selected, representing potentially different EDC exposure scenarios. The first site is viewed as an exposure model for pesticide and other organic EDCs, whilst the second site is representative of high inorganic EDC exposure hazards. The third site was viewed as a control site. The second site is well-placed to serve as a baseline exposure site for inorganic EDCs sourced from groundwater, a critical factor for local rural communities presenting with similar geochemistry. The importance of such exposures in the National context across South Africa was detailed in the WRC report (1175/1/06) [69] wherein the majority of over 600 new subterranean sources drilled by the Department of Agriculture (2005-2008) presented with potential inorganic EDCs at concentrations exceeding most local and international guidelines based on toxicity. This site could thus serve as additional inputs towards motivating for similar studies applicable to public health within the relevant communities.

Site 1:

The baseline water quality investigation returned marginal risk for inorganic EDC hazards. Water provision is from a single abstraction point in the Komati River. This system is well characterized elsewhere and generally regarded as a severely impacted river system as it receives potential runoff from agricultural chemicals in the form of pesticides from sugar-cane and citrus plantations and

settlements upstream and thus a potential source of potential organic EDCs (consult WRC Project K5/1956 for additional pesticide information). The housing types are open-sided (as opposed to closed with controlled ventilation) with commercial sugar-cane and citrus plantations immediately adjacent and the system is a multi-age system (compared to all-in all-out), with specimens available at the maximum exposure age all year round.

Site 2:

The baseline water quality investigation indicated high exposure concentrations to inorganic EDCs associated with the Bushveld Igneous Complex in the Rustenburg District. This region was investigated in terms of inorganic water quality for both subterranean and surface water sources for nearby rural communities, specifically within the Jericho district (WRC Report 1175/1/06) and the Pilanesberg National Park (SANParks water quality investigations reported in WRC Reports 644/1/98 & 857/1/01). Additional evidence of inorganic water quality constituents as naturally occurring geochemical anomalies throughout South Africa at potentially hazardous concentrations in rural communities and commercial agriculture projects is presented in the WRC Report 1175/1/06 [69]. This site thus represents an ideal sentinel platform from which to address the EDC effects from inorganic constituents that typically draw focus to classic toxicological effects, predominantly with reference to nitrate, fluoride and numerous trace elements. The housing system employed is closed and environmentally controlled in terms of heat and ventilation.

Site 3:

This represents a commercial operation located in the Gauteng Province selected due to the use of municipal water on two adjacent sites for which water quality tests conducted had revealed very low to zero exposure to inorganic EDCs. No disinfection is applied on site and residual chlorine levels tested at <0.2 ppm. The system is similar to that for site 2.

4.6.3 Brief Overview of Methods

The following samples were obtained (refer to Deliverable 6 for WRC Project K5/1915 for full detail):

Overview

• **Water** Quality for Source, Storage and Point of Use:
  o Full inorganic chemistry
  o Microbiological indicator organisms
  o Oestrogenic and Androgenic bio-assays
  o Variable Physico-chemistry (site dependent)
 	
• **Tissue** Samples:
  o 10 specimens per site (all 35 days of age and collected from the three sites within a 60 day period):
  ▪ Thymus (bilateral) – Histopathology
  ▪ Thyroid & parathyroid (bilateral) – Histopathology
  ▪ Bursa of Fabricius – Histopathology

Overview

- Gonads (Testes) – Histopathology
- Liver – inorganic trace ICP-MS
- Deep Pectoral – inorganic ICP-MS
- Venous blood (brachial vein) – whole blood for thyroxine (T4)

  - Evaluation of each specimen for gross macroscopic lesions

- **Sediment** samples for Oestrogenic, Androgenic and Anti-androgenic bioassays

Water Detail

Water samples were submitted to the ISCW at the ARC. Due to the subsequent development of the PAL at NECSA methodology for full quantitative ICP-MS tests (refer to WRC K8/999) the last sampling phase entailed submission of samples to PAL and ISCW in order to compare results (notably for Set B samples).

Microbiological assessments were performed by the Food & Microbiological Section at the South African Bureau of Standards in accordance with SANS 241:2006 (Edition 6.1) (SANS 5221: 2007 Ed 4.3 & K.M.5.4W-B-TC & EC).

The physico-chemical parameters were conducted by the Water Chemistry Section at the South African Bureau of Standards in accordance with SANS 241:2011 (Aquakem-colimetric – ammonia; SANS 5213: 2005 – COD; SANS 6049: 2004 – SS; SANAS TOO8 – Surfactants).

Water samples were also collected for the assessment of both oestrogenic and androgenic activity via bioassays. The following tests were performed:

- Assessment of Oestrogenic Activity using the Recombinant Yeast Oestrogen Assay and the T47D-KBLUC assay
  - Performed by the Department of Urology, Steve Biko Academic Hospital, University of Pretoria.

- Assessment of Androgenic Activity using the MDA-kb2 cell line
  - Performed by the School for Environmental Sciences and Development, Faculty of Natural Sciences at the North West University.

Biological Tissue Detail	
A total of 10 Ross 308 male broilers from each site were collected at day 35 in the production cycle and sacrificed by cervical dislocation. The following table presents the sample collection methods and tests performed.	
Sample Type	**Test Performed**
Whole Blood	Collected from the Brachial Vein in lithium heparinized vacutainers (LH BD Vacutainer 4 mL) from live bird. Tested for plasma thyroxine (Total T4). Submitted to: Veterinary Hormone Laboratory Faculty of Veterinary Science University of Pretoria
Tissues: Thyroid (bilateral) Thymus (bilateral – poultry) Bursa of Fabricius (poultry) Testis (male broilers)	**Histopathology:** Samples were immediately fixed in 10% buffered formalin and submitted to: Department of Paraclinical Sciences Faculty of Veterinary Science University of Pretoria
Tissues: Hepatic (whole) Supracoracoid muscle (poultry)	**Inorganic EDC determination**: The same profile of inorganic assessments for clinical biochemistry differential diagnostics as detailed for the inorganic water samples was performed using ICP-MS: Submitted to the ISCW (ARC).

The specific methods for the ICP-MS determination are presented in WRC Report K8/999 but briefly involve a multi-element microwave assisted acid digestion of the tissues prior to analysis by the ICP-MS method also described in the WRC report referred to.

Ten specimens of the same age and conformity were obtained from each Site with duplicates being prepared for each tissue type (thus 20 liver and 20 breast samples analysed per specimen). The arithmetic mean of each duplicate was then used as the individual specimen value with pooled median values used as site observations. This technique is used for determining clinical biochemistry trends in investigating nutritional pathologies. Although this was not the emphasis of this investigation such interpretations may be performed such it be required.

The goal of this method is total sample decomposition with acids. A benefit to this method is that the same ICP-MS apparatus is used for water, sediment and biological tissues with a significant benefit in reducing inter-laboratory variation. This is advantageous from a monitoring perspective.

Sediment Samples

Not all of the three sites in Set A were subjected to the same procedures. Sediment samples were only collected for Set A Site 1 as the source for drinking water was abstraction from the Komati River. Sites 2 and 3 utilise groundwater and municipal water respectively so no sediment samples were relevant. The same procedure was however used for Set A Site 1 and surface water sampling sites for Set B.

Composite sediment samples were collected and transported in high density poly-ethylene bottles (Nalgene). Three 500 ml bottles were collected at each site after being acid-rinsed. These were pooled with two 250 ml bottles being extracted. Samples were stored at -20°C and submitted to the School for Environmental Sciences and Development, Faculty of Natural Sciences at the North West University for the extraction protocol.

There the frozen samples are subsequently thawed and air dried at room temperature whilst being protected from ultraviolet radiation break down. Dried sediment is then ground and sieved (0.5 mm mesh size) to obtain homogenous samples from which an extraction process is commenced.

Full methods are presented in the Water Research Commission Project: K5/1956/Deliverable 6 Chapter 3 for the androgenic assays, but the extractions are then used for both the oestrogenic and androgenic bioassays.

The following tests were performed on the extracts:

- Assessment of Oestrogenic Activity using the Recombinant Yeast Oestrogen Assay and the T47D-KBLUC assay
    - Performed by the Department of Urology, Steve Biko Academic Hospital, University of Pretoria.

- Assessment of Androgenic Activity using the MDA-kb2 cell line
    - Performed by the School for Environmental Sciences and Development, Faculty of Natural Sciences at the North West University.

4.6.4 Results

4.6.4.1 Water Quality Results

For the purposes of this volume only the key observations are presented (for full results and assessments refer to Deliverable 6 for K5/1915). The sites for Set A are routinely monitored as part of the biosecurity and health care programme by specialist poultry veterinarians. No significantly adverse microbiological indicator organism counts were observed although elevated Total Plate Counts were noted in the drinker lines (frequently observed due to the in-house environmental conditions being conducive to microbial growth). These values are in themselves more process

indicators than health risk indicators and thus not reported in great detail here. The following key results were observed:

Site 1:

Key Inorganic Chemistry values observed (mg/L)

Samples	**Water Quality Constituent** (mg/L)				
	Total Dissolved Solids	**Iron**	**Bromide**	**Total Hardness**	**Anomalies**
Komati River	**80**	**1.071**	**0.656**	51	Mn = 0.105 Pb = 0.025
Source Water	180	0.099	**0.498**	61	
Point of Use	177	0.525	**0.610**	61	Cu = 0.234
Komati River Tributary	478	0	**0.201**	317	Sr = 0.298
Komati River Phase 2	256	0.969	**0.150**	161	Sr = 0.159
Point of Use Phase 2	226	1.02	**0.154**	117	Sr = 0.154
pH values [8.09 7.92 7.94 7.95 7.96]					

Microbiological values observed

Sample	**Water Quality Indicator Organisms**		
	Total Bacteria Count (Count/mL)	**Coliform Bacteria Count** (Count/100 mL)	***E. coli*** (count/100 mL)
Komati River Phase 2	33	0	0
Point of Use Phase 2	> 30 000	0	0

Key Physico-chemical values observed (mg/L)

Samples	**Water Quality Parameter**			
	Ammonia (as N mg/L)	**Chemical Oxygen Demand** (mg/L)	**Suspended Solids** (mg/L)	**Surfactants** (mg/L)
Komati River Phase 2	<0.3	<10	<10	<10
Point of Use 2	<0.3	<10	<10	<10

Site 2:

Key Inorganic Chemistry values observed (mg/L)

Samples	**Water Quality Constituent** (mg/L)				
	Total Dissolved Solids	**Nitrate**	**Bromide**	**Total Hardness**	**Anomalies**
Point of Use	**1529**	**98**	**0.942**	**1247**	Sr = **1.207;** Mg = 175
Point of Use Phase 2	**1379**	**92**	**1.920**	**951**	Sr = **2.083;** Fe = 0.05 Hg = 0.026
Borehole Source 1	**1419**	**94**	**2.029**	**1086**	Sr = **1.564;** Fe = 0.23 Hg = 0.014
Borehole Source 2	**1338**	**93**	**1.62**	**873**	Sr = **1.286;** Fe =0.14 Hg = 0.005

Point of Use Phase 3	**1488**	**55**	**3.280***	**1047**	Sr = **2.100*;** Fe = 0.035; NO_2 = 15
Borehole Source 1 Phase 2	**1400**	**92**	**2.870***	**1010**	Sr = **2.520*;** Fe = 0.05; NO_2 = 7
Borehole Point of Use Phase 4	**1488**	**92**	**2.870**	**1047**	Sr = **2.152;** Fe = 0.05; NO_2 = 7 Se = 0.163; F = 1.37
New Borehole 1 Source Phase 4	**1211**	**100**	**1.751**	**949**	Sr = **1.605;** Fe = 0.04; NO_2 = 5 **Pb = 0.074**; F = 1.42
New Borehole 2 Source Phase 4	**552**	**3**	**0.081**	**494**	Sr = 0.803**;** Fe = 0.01; NO_2 = 1 **Pb = 0.011**; F = 0.92
pH values = [7.25 8.13 7.86 8.37 6.78 7.24] * =Submitted to PAL for full quantitative ICP-MS to validate Br and Sr results.					

Site 3

Key Inorganic Chemistry values observed (mg/L)

Samples	**Water Quality Constituent** (mg/L)				
	Total Dissolved Solids	**Nitrate**	**Bromide**	**Total Hardness**	**Anomalies**
Municipal Source	133	1	0.043	102	Fe = 0.253 Sr = 0.074
Point of Use	132	2	0.017	80	Fe = 0.332 Zn = 1.134; Sr = 0.093
pH values = [8.31 8.38]					

Microbiological values observed

Sample	**Water Quality Indicator Organisms**		
	Total Bacteria Count (Count/mL)	**Coliform Bacteria Count** (Count/100 mL)	***E. coli*** (count/100 mL)
Municipal Source	1	1	0
Point of Use	24 450	12	3
Municipal Source Phase 2	3	1	0
Point of Use Phase 2	>30 000	6	2

4.6.4.2 Thyroid Observations:

Table 25. Set A TH. Comparisons between observed data and reference values for plasma Thyroxine (total T4).

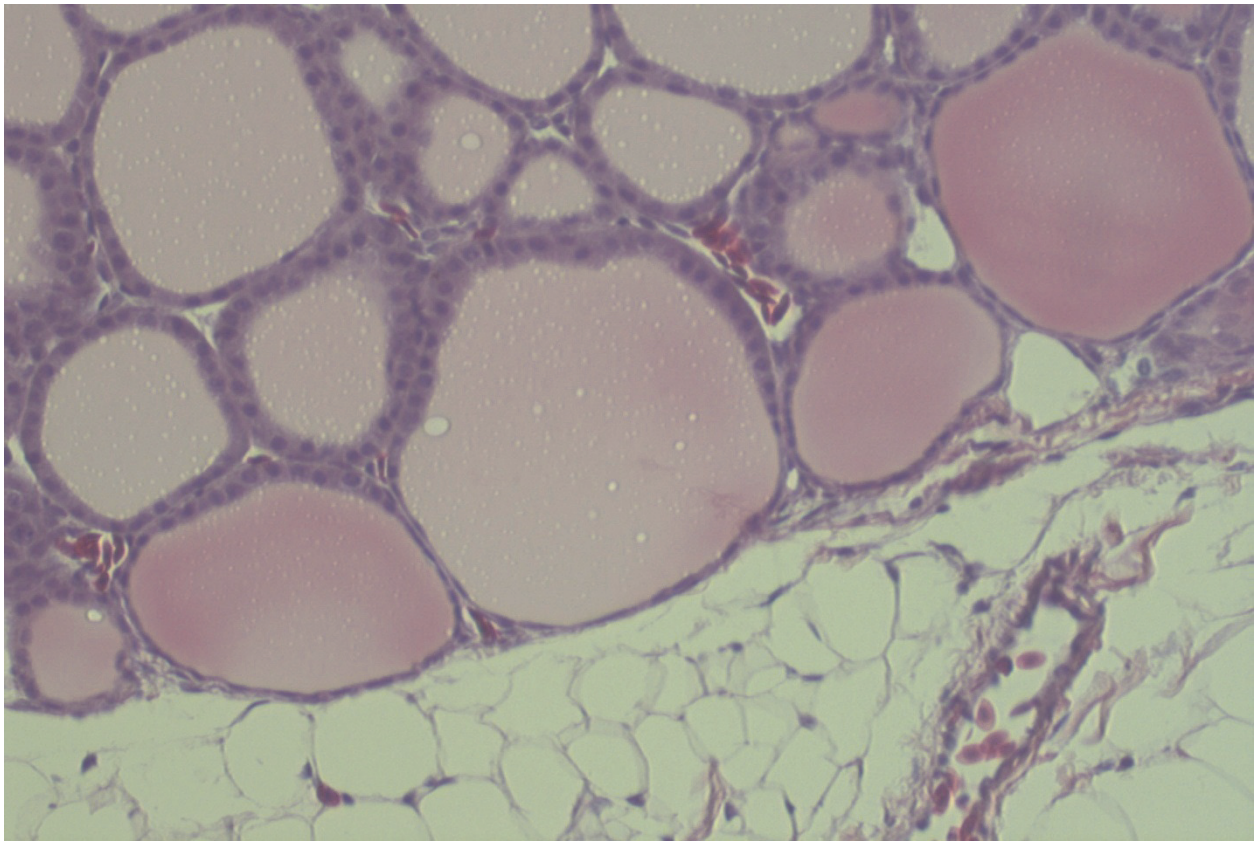
Plasma Thyroxine values (Total T_4) in nmol/L at 35 days (median ± SD)					
Observed Sites* (n= 10 per site)			**Reference: Ross^**	**Reference: Non Commercial^**	**Reference: Range for 6 commercial broiler breeds^**
Site 1	**Site 2**	**Site 3**			
[a]3.14 ± 1.696	[a] 4.945 ± 1.625	[b] 9.855 ± 4.098	10.89 ± 0.849	8.906 ± 1.081	9.715 ± 0.514 10.707 ± 0.489
Plasma Thyroxine values (Total T_4) in nmol/L at 1 day					
Reference: Ross^		**Reference: Non Commercial^**		**Reference: Range for 6 commercial broiler breeds^**	
5.933 ± 0.785		5.997 ± 0.123		4.337 ± 0.694 6.151 ± 0.939	

^ Adapted from Gonzales et al. (1999) n = 12 per breed. [71]

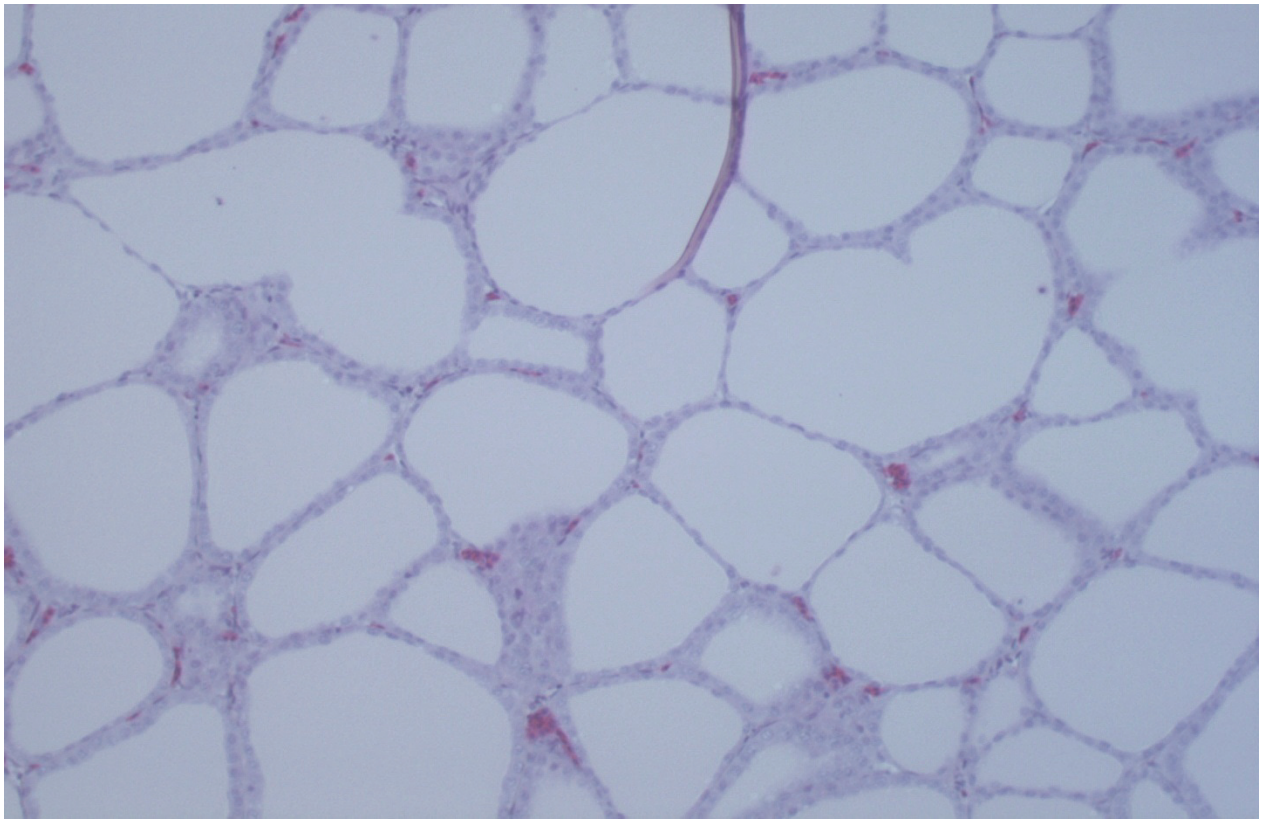
*Values without a common superscript differ significantly (P≤0.05)

The following histopathology slides correspond to the Sites as follows: Slide 1 = Site 3, Slide 2 = Site 2, Slide 3 = Site 1:

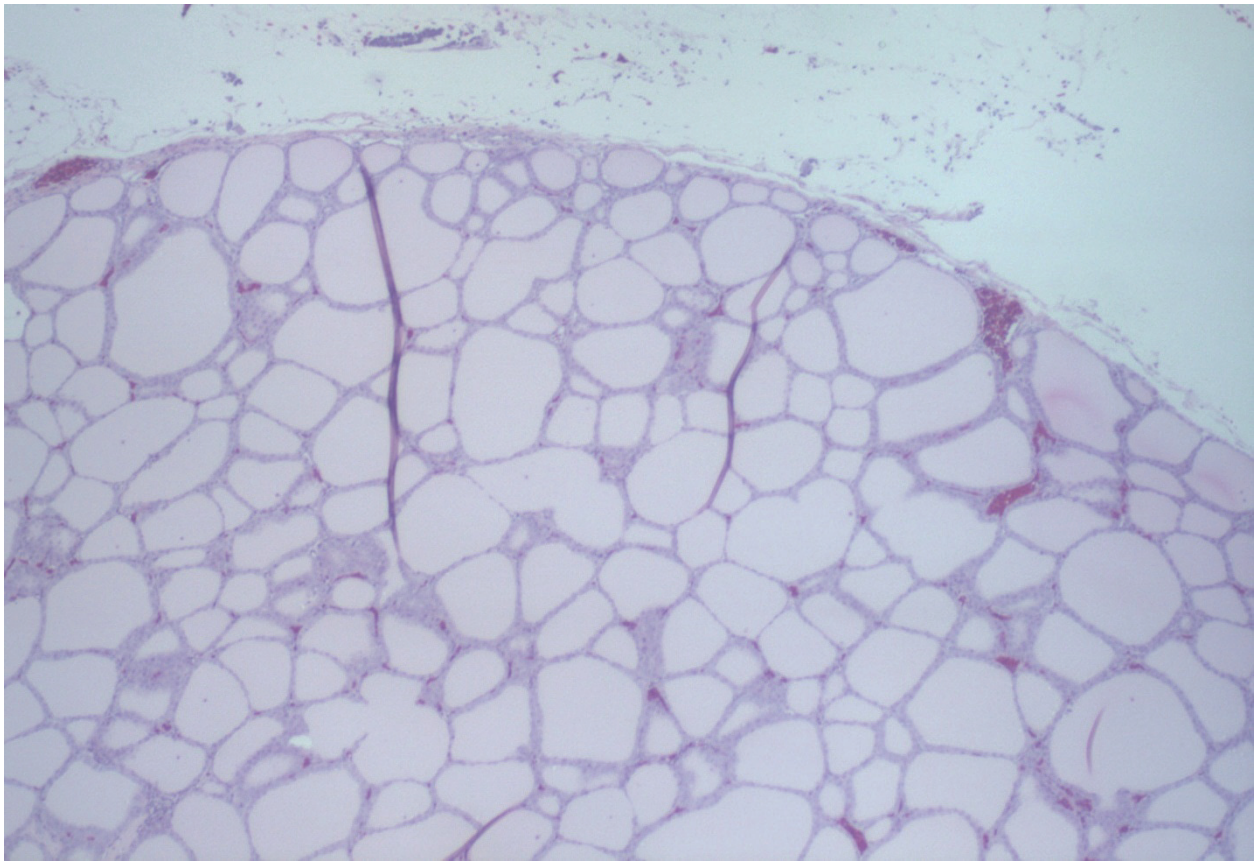
Slide 1: Site 3



Slide 2: Site 2



Slide 3: Site 1



4.6.4.3 Tissue Observations

Due to the comprehensive list of elements determined full results are not presented here, but key observations for the elements relevant to EDC effects observed are.

Table 26. RT1. Key Deep Pectoral Tissue values observed (mg/kg FW).

		Site Values*		
Element	**Median Standard Deviation [Range]**	**Site 1**	**Site 2**	**Site 3**
As	*Md*	0.004	0.041	0.415
	s	0.006	0.015	0.061
	[ ]	[0.001-0.16]	[0.01-0.062]	[0.021-0.23]
Br	*Md*	1.942	12.514	1.921
	s	1.384	4.264	5.323
	[ ]	[1.624-6.063]	[5.019-18.645]	[1.39-13.165]
Se	*Md*	0.077	0.226	0.16
	s	0.017	0.062	0.074
	[ ]	[0.055-0.111]	[0.11-0.339]	[0.058-0.316]
I	*Md*	0.006	0.023	0.001
	s	0.009	0.013	0.010
	[ ]	[0.004-0.028]	[0007-0.051]	[0.001-0.031]
Sr	*Md*	0.031	0.02	0.024
	s	0.023	0.185	0.017
	[ ]	[0.021-0.096]	[0.015-0.61]	[0.008-0.048]
Pb	*Md*	0.001	0.02	0.003
	s	0.008	0.018	0.021
	[ ]	[0.001-0.025]	[0.001-0.06]	[0.001-0.061]
Cu	*Md*	0.246	0.252	0.385
	s	0.054	0.564	0.049
	[ ]	[0.161-0.337]	[0.05-11.811]	[0.311-0.453]
Mn	*Md*	0.097	0.147	0.183
	s	0.016	0.027	0.039
	[ ]	[0.017-0.129]	[0.118-0.203]	[0.118-0.264]
Zn	*Md*	6.035	7.519	6.233
	s	0.881	1.963	0.863
	[ ]	[5.292-7.415]	[5.528-12.485]	[5.23-7.577]

* values pooled duplicates from n = 10 per site (20 determinations per specimen sample type)

Table 27. RT2. Key Liver Tissue values observed (mg/kg FW).

		Site Values*		
Element	**Median Standard Deviation [Range]**	**Site 1**	**Site 2**	**Site 3**
As	*Md*	0.019	0.052	0.046
	s	0.005	0.008	0.01
	[ ]	[0.009-0.026]	[0.044-0.071]	[0.02-0.061]
Br	*Md*	4.461	19.438	5.617
	s	0.624	3.996	6.864
	[ ]	[3.342-5.304]	[14.265-25.405]	[0.361-18.66]
Se	*Md*	0.456	0.622	0.495
	s	0.041	0.045	0.109
	[ ]	[0.365-0.491]	[0.532-0.679]	[0.354-0.65]
I	*Md*	0.043	0.034	0.001
	s	0.007	0.043	0.073
	[ ]	[0.03-0.053]	[0.012-0.133]	[0.001-0.24]
Sr	*Md*	0.045	0.042	0.031
	s	0.011	0.008	0.015
	[ ]	[0.03-0.053]	[0.028-0.052]	[0.015-0.055]
Pb	*Md*	0.001	0.215	0.001
	s	2.2E-19	0.009	0.007
	[ ]	[0.001-0.001]	[0.011-0.037]	[0.001-0.023]
Cu	*Md*	2.532	3.649	2.745
	s	0.321	2.598	0.418
	[ ]	[1.854-3.05]	[2.069-10.05]	[2.018-3.309]
Mn	*Md*	2.117	2.482	1.988
	s	0.224	0.477	0.250
	[ ]	[1.78-2.5]	[1.572-2.892]	[1.437-2.308]
Zn	*Md*	17.452	28.54	21.13
	s	3.794	5.482	10.716
	[ ]	[14.035-28.22]	[24.38-42.185]	[15.315-44.84]

* values pooled duplicates from n = 10 per site (20 determinations per specimen sample type)

Table 28. RT3. Consistent Tissue values observed between Sites (mg/kg FW).

Element	**Median Deep Pectoral Values***			**Median Liver Values***		
	Site 1	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
Cd	0.001	0.001	0.003	0.007	0.007	0.007
Co	0.001	0.001	0.003	0.021	0.01	0.026
Cr	0.042	0.052	0.045	0.039	0.055	0.04
Mo	0.056	0.091	0.097	0.460	0.576	0.605
Ni	0.001	0.001	0.050	0.001	0.007	0.036
Hg	0.001	0.004	0.007	0.018	0.007	0.016
V	0.012	0.026	0.017	0.049	0.055	0.195
U	0.001	0.001	0.001	0.001	0.001	0.001

* values pooled duplicates from n = 10 per site (20 determinations per specimen sample type)

4.6.4.4 Bioassays

4.6.4.4.1 MDA-kb2 Reporter Gene Bioassay

This assay was used to determine the androgen activity of water and sediment samples. Since Sites 2 and 3 utilise subterranean sourced water distributed to the production site no relevant sediment sample sites were applicable. The results obtained for Set A are presented below.

Table 29. RB1. Summary of Androgenic Bioassay responses elicited in Water samples for Set A.

Set A Samples	**Activation of Androgen Receptor**	**Inhibition of Androgen Receptor (REP20)* µg F-eq/L**	**Cytotoxicity**
Site 1: Komati River	No	1304.11 ± 3.74	No
Site 1: Point of Use	No	No	No
Site 2: Borehole Source	No	No	No
Site 2: Point of Use	No	No	Yes
Site 3: Point of Use	No	No	No

* Relative effects potencies (REP 20-80) ratio between EC20-80 values of the reference control and samples submitted expressed as flutamide equivalents.

Of relevance to these results and those reported for the androgen bioassays conducted for Set B water and sediment samples are the limits of detection (LOD) calculated for each matrix type. The observed values were:

Sediment LOD:	**Water LOD:**
2.5 ± 0.5 pg T-eq/g 1.59 ± 0.86 µg F-eq/g	41.77 ± 1.15 µg T-eq/L 15.93 ± 0.04 g F-eq/L

Table 30. RB2. Summary of Androgenic Bioassay responses elicited in Sediment samples for Set A.

Set A Samples	**Activation of Androgen Receptor (REP20)* ng T-eq/g sediment**	**Inhibition of Androgen Receptor**	**Cytotoxicity**
Site 1: Komati River Upstream	15.63 ± 3.74	No	Yes
Site 1: Komati River Abstraction point	6.40 ± 1.25	No	No

* Relative effects potencies (REP 20-80) ratio between EC20-80 values of the reference control and samples submitted expressed as bio-assay derived equivalents.

4.4.6.4.2 Yeast Estrogen Screen (YES) and T47D-KBluc Reporter Gene Bioassays

These assays were used to determine the oestrogenic activity of water and sediment samples. Since Sites 2 and 3 utilise subterranean sourced water distributed to the production site no relevant sediment sample sites were applicable.

The YES assay identifies compounds that can interact with the human oestrogen receptor alpha (ERα) [72], whereas the T47D-KBluc assay was developed by the US EPA using human breast cancer cells which contain both endogenous ERα and ERß providing an *in vitro* system to evaluate the ability of chemicals to modulate the activity of oestrogen-dependent gene transcription [73]**.** The results obtained for Set A are presented below.

Table 31. RB3. Summary of the YES bioassay responses in Water samples for Set A.

Set A Samples	**YES assay***	**Cytotoxicity**
Site 1: Komati River	No oestrogenic activity detected	None reported
Site 1: Point of Use	No oestrogenic activity detected	None reported
Site 2: Borehole Source	No oestrogenic activity detected	None reported
Site 2: Point of Use	No oestrogenic activity detected	None reported
Site 3: Point of Use	No oestrogenic activity detected	None reported

* Activity expressed as oestrodial equivalents interpolated from standard oestradial curve.

Table 32. RB4. Summary of the YES bioassay responses in Sediment samples for Set A.

Set A Samples	**YES assay***	**Cytotoxicity**
Site 1: Komati River Upstream	No oestrogenic activity detected	None reported
Site 1: Komati River Abstraction point	No oestrogenic activity detected	None reported

* Activity expressed as oestrodial equivalents interpolated from standard oestradial curve.

Table 33. RB5. Summary of the T47D-KBluc bioassay responses in Water samples for Set A.

Set A Samples	T47D-KBluc assay* EE(ng/L)	Cytotoxicity
Site 1: Komati River	0.058 ± 0.016	None reported
Site 1: Point of Use	0.071 ± 0.003	None reported
Site 2: Borehole Source	0.013 ± 0.002	None reported
Site 2: Point of Use	Below detection limit^	Yes
Site 3: Point of Use	0.016 ± 0.003	Yes

* Activity expressed as oestrodial equivalents interpolated from standard oestradial curve.

^ Note cytotoxicity can mask oestrogenic activity.

Table 34. RB6. Summary of the T47D-KBluc bioassay responses in Sediment samples for Set A.

Set A Samples	T47D-KBluc assay* EE(ng/L)	Cytotoxicity
Site 1: Komati River Upstream	No oestrogenic activity detected	None reported
Site 1: Komati River Abstraction point	No oestrogenic activity detected	None reported

* Activity expressed as oestrodial equivalents interpolated from standard oestradial curve.

4.6.5 Discussion Set A

Water Quality

Inorganic Quality
<u>General</u> The water quality observed for Site 2 would be classified as not suitable for broiler production due to the following hazards: - **high nitrate** (methemoglobinemia) - **high bromide** (endocrine disruption & disinfection byproducts & exacerbated by high strontium) - high total hardness (scaling of water distribution system)

Inorganic Quality

These hazards may result in adverse effects on toxicological, palatability and watering system norms and may also result in endocrine disruption – refer to Thyroid results later. Although Site 1 did present with some inorganic hazards the risks were marginal when compared to Site 2, with the primary suspected constituents responsible most probably related to the organic and pesticide concerns within the catchment. The use of Site 3 as a reference site was supported by the low values noted for inorganic EDCs.

In general numerous additional issues relating to production and water quality were also applicable but are beyond the scope of this volume too address here with the exception of the presence of bromide in most groundwaters resulting in a significant hazard for the formation of carcinogenic bromate with the use of chlorine as routine disinfectant. Since the use of chlorine as disinfectant for continuous and intermittent applications in the broiler industry is widespread recognition of this hazard, particularly for breeder farms, should be highlighted. A similar concern exists for nitrate and reactions with disinfectant chemicals.

Microbiological Quality

General

Some of the Total Bacteria Counts observed, although higher than the desired limit, do not represent a health hazard, but rather demonstrates that the water quality conditions are conducive to the establishment and growth of micro-organisms. This is often not correctly interpreted with the reaction tending towards increased application of disinfectant chemicals. Concern, as noted in the previous table for inorganic chemistry, exists of the lack of recognition of the formation of disinfection byproducts influenced by the inherent chemistry, which may be endocrine disruptive.

A similar situation occurs with regards to the Coliform Bacteria Count and presence of *E. coli* which, although undesirable, are also only indicator organisms that should result in specific tests for suspected pathogenic organisms when indicated by health investigations and related problems, prior to the application of increased disinfection levels.

Thus although also in excess of the preferred limits they tend to still only be marginal hazards but often result in higher application doses of disinfectant chemicals. It has been noted by the DOA that bromide residues due to the use of fumigants containing methyl bromide have been noted in the commercial sector. This aspect also warrants more education within the agricultural sector.

Physico-chemical Properties

General

Although only determined for one sampling phase for Site 1 the results were at a seasonal observation point where little runoff and pesticide application was expected. At the time of sample submission the pesticide screens initially intended were not yet validated.

A separate WRC Project (K5/1956) is currently underway with pesticide and other organic testing being conducted at this site and elsewhere in the catchment. This includes bioassays and air quality monitoring for pesticides, a key suspected route of exposure for the sentinel model at this site.

Thyroid observations

The observed plasma T4 values for Sites 1 and 2 were significantly lower than those observed for the control Site 3 and indicative of an integrated result of secretion, biological use and elimination. The control site values accorded with the reference values available, whilst those for Sites 1 and 2 were suggestive of hypothyroidism and supported by the histopathology noted for the thyroid sections. These sections presented with low to absent staining of colloid for Sites 1 and 2 with low to absent vacuolation. The intensity of staining and resorptive capacity for the control Site 3 was normal and supportive of the higher T4 values observed in plasma.

Although there are numerous pathways where EDC action may disrupt thyroid synthesis, storage, release and action at target tissues, the objective of the case study sites was not to describe the mechanisms relevant, but to identify endocrine disrupting activity that would serve to initiate more detailed investigations. Despite this the observations do suggest a fundamental disruption at the synthesis stage, most probably involving the thyroxine biosynthetic pathway with both bromide and selenium involved. Additional TSH pathway disruption may also apply with reference to follicular cell membrane vesicle formation.

Gonzales *et al.* (1999) [71] reported plasma thyroxine values for various strains and ages of broilers with a comparison for the same age and breed as collected for this study presented in the results section and noted that the modern broiler strains were slightly hypothyroid when compared to the Naked Neck strain (indigenous breed) and concluded that the selection pressures for growth and efficiency may have led to altered thyroid hormone metabolism which may increase susceptibility to SDS and AS. Some functional hypothyroidism may be indicated by low T3 and unchanged T4, but in the case of the Naked Neck strain higher circulating T3 and lower T4 values were found at similar ages, supposedly due to the “slightly hyperthyroid nature” in this strain when compared to commercial

Thyroid observations

strains. This should be noted when collecting sentinel specimens in rural settings compared to commercial breeds.

In the rural breeds the lower plasma T4 values were due to GH inhibition on T3 degrading Type III hepatic activity and subsequent negative feedback by T3 on the hypothalamo-hypophyseal-thyroid axis [74]. In the study by Gonzales *et al*. (1999) [71] the 6 commercial broiler strains tested were very similar in the thyroid hormone endocrine profiles (comparative means provided in results table also).

Whilst T3 values were not determined this would be indicated if more specific investigation into the mechanisms involved were desired. The current values obtained suggest that the T4 values were sufficiently reduced enabling a diagnosis to be made given the normality of the observed specimen groups and extent of lowered values obtained compared to the reference values and control site values. It has to be considered that for other case studies the observed T4 values may be lower but not clearly indicative of a physiological significant consequence. The observation of endocrine disruption is in itself not necessarily an adverse effect, but should the observed T4 values be marginal or only suggestive of disruption with potential physiological significance then it would be recommended to not only conduct the histopathology as performed for these sites, but to also include T3 in order to establish the T_3:T_4 ratio as an additional diagnostic tool [75].

Thus, although it may seem a simple task to thus translate endocrine disruption to a physiological significance the numerous management factors relevant often render differential diagnosis thereof complex. The combination of EDC exposure media data, blood hormone values and histopathology are thus recommended to contribute towards confidence in both diagnostics and the decision to accord priority for further investigation to specific sites.

Tissue and Bioassay observations Summary

The tissue values for inorganic constituents were supportive of the thyroid hormone observations and the histopathology evaluation. The following key observations refer:

	Site 1	Site 2	Site 3	Reference Value
Hormone values (nmol/L):				
Total Thyroxine	**3.14**	**4.945**	9.855	10.89 (35 days) 5.93 (1 day)
Point of Use PHC (mg/L):				
Phase 1 Bromide	0.154	**0.942**	0.017	0.01 (US EPA MCL)
Phase 2 Bromide	0.650	**3.280**	0.043	
Bioassays – water:				
Androgenic:				
Inhibition	**yes**	no	no	
Oestrogenic:				
Activation	**yes**	**yes**	**yes**	
Cytotoxicity	no	no	**yes**	
Bioassays – sediment:				
Androgenic:				
Activation	**yes**	no	no	
Cytotoxicity	**yes**	no	no	
Oestrogenic:				
Activation	no	no	no	
Cytotoxicity	no	no	no	
Tissue Values (mg/kg FW):				
Deep Pectoral:				
Bromide	1.942	**12.514**	1.921	
Selenium	0.077	**0.226**	0.160	
Iodine	0.006	**0.023**	0.001	
Lead	0.001	**0.03**	0.003	
Liver:				
Bromide	4.461	**19.438**	5.617	
Selenium	0.416	0.622	0.495	
Iodine	0.006	**0.023**	0.001	
Lead	0.002	**0.215**	0.001	

Both Sites 1 and 2 provided evidence of low blood T_4 values, with the higher bromide exposure for Site 2 reflected in hepatic tissue yielding higher total values but pectoral tissues reflecting greater deviation between comparison sites. The higher selenium values for Site 2 for both tissue types and isolated higher values (e.g. iodine breast and manganese liver) may be due to complexing as similar observations have been reported in other WRC projects investigating risk assessment of groundwater for humans and livestock as a primary

mechanism by which bromide exerts its effect on thyroid function (WRC Report No. 1175/1/06). The increased selenium noted for pectoral and liver tissues for Site 2 may be related to a primary mechanism in non-ruminants involving to a membrane-bound selenoprotein (type 2 iodothyronine deiodinase) that is capable of transforming the T_4 to the physiologically active form, T_3 (refer to Arthur and Beckett 1999 for a review) [76]. Follow up sampling for Site 2 to investigate two new potential boreholes yielded some hazardous selenium and lead anomalies. Similar values were recorded in rural communal villages within the same area for other WRC studies. The variation between subterranean sources within close proximity is well documented and supports the need for site-specific investigations and increased monitoring to establish variation over time.

The increased hepatic iodine noted for Site 1 and 2 accords with the low T_4 values observed, and within the context of all sites receiving high biological availability formulated feeds, these sites and the issues regarding complexing and availability appear to be significant.

The interpretation of the induced deficiencies accord with the EDC type of effect in which neither direct toxicity or adverse effect is necessarily involved, but rather disruption at the cellular level of, in this case, synthesis. This is not that dissimilar to some iodine deficiency disorders where goitrogenic substances effectively increase the dietary requirement for iodine despite the actual iodine intake being supposedly sufficient (in the absence of goitrogenic substances). These similar observations noted higher values in renal cortex tissues suggestive that the primary adverse effect was to lower the available selenium and manganese (and possibly other trace elements) required for thyroid function (iodine activation) with corresponding high values noted in renal tissues. Further detail may be obtained from Arthur *et al.* (1992) [77] and Beckett and Arthur (1994) [78].

The observation of a beneficial response on supplementation of these elements was noted in previous studies in production animals (WRC Report No. 1175/1/06) with whole blood values thus not viewed as reliable indicators of status.

There are other links to broiler-related production problems that involve thyroid function and potential endocrine disruption. Although not reported on in this report some of the post mortems conducted for Site 2 presented with Tibial dyschondroplasia (TD) which is a leg defect with a lesion of avascular, non-calcified cartilage below the growth plate of the proximal tibiatarsus. This is due to the inability of chondrocytes to undergo terminal differentiation, and since thyroid hormones are required for chondrocyte differentiation a potential link to the observations made could be explored. Refer to Shen *et al.* (2004) for further detail [79].

Summary

The effects on blood thyroid values for Site 2 appear linked to the high exposure values to inorganic PHCs, notably bromide. For Site 1 however the effects are most probably linked to other types of EDCs as the bromide values in the tissue types collected did not appear different to those of the control site. The primary candidate EDC type for Site 1 is possibly influenced by pesticide exposure (open-sided ventilation houses with production crops surrounding broiler houses) and is part of a separate WRC project (K5/1956). This statement would appear to be in part supported by the bioassay results obtained for the Komatipoort river and sediment samples. In addition the WRC Project No. 1956 has awarded a high risk priority to some pesticides containing bromide in the South African context.

Bromide use in environmental applications has been subject to numerous reviews and amendments in terms of registrations, restrictions and removal of restrictions. Although too detailed to present here it is noteworthy that derogations are argued for many African countries where food security is deemed more essential than protection of the environment from pesticide residues. Further reading may be obtained from the US EPA (http://www.epa.gov/oppsrrd1/registration_review/bromine/index.htm and http://www.bromine-info.org/en/Bromine-Applications/).

In an experiment comparing 2,4-D, simazine and bromide applications to crops in terms of leaching and runoff, it was observed that bromide moved more readily in the soil profile than simazine which tended to remain in the surface soil. It was also noted that 2,4-D took 24 days to dissipate from the soil [80].

A recent cancellation order was issued by the US EPA (May 2011) for the termination of the use of methyl bromide in soil uses. It is noteworthy that none of the registrants chose to respond to the earlier (February 2011) request for comments to be considered prior to the cancellation order EPA-HQ-OPP-2005-0123.

The United Nations Environment Programme focusing on the phasing out of methyl bromide notes that in South Africa the primary usage sector is listed as the production of grapefruits, tobacco, flower, strawberry, apples and vegetables (http://www.unep.org/roa/Projects_Programmes/Ozone/MB/Projects/South_Africa.asp).

The UNEP programme notes the key uses as being:

- Soil:
  - Pre-plant and propagation treatment as insecticide and herbicide

    - Replant treatment (prevention of replant disease)
    - Fungicide (notably for tobacco)
- Durables:
    - Insecticide for cereal grain pests and similar commodities in storage and trade (including export, import as quarantine).
    - Insecticide and fungicide for timber and wooden pallets.
- Perishables:
    - Insecticide for phytosanitary or quarantine treatment in fresh fruit, vegetables and cut flowers.
- In structures and transport:
    - Insecticide treatment for food facilities, flour mills and related buildings.

Notable exemptions listed for this phase-out programme include uses for quarantine, pre-shipping and the critical use exemption clause being used in instances where no technologically and economical alternative is feasible and where withdrawal will result in significant market disruption. The presence of bromide is by no means limited to methyl bromide with a compendium on bromine in pesticides noting that a total of 59 substances contain bromine, ranging from a variety of fungicides, nematicides, molluscicides to organochlorine insecticides, pyrethroid ester insecticides, quaternary ammonium herbicides and organophosphate acaricides and insecticides. A full list is available from http://www.alanwood.net/pesticides/index-haic-br.html.

Some of these are noted in the list of pesticides registered for use in South Africa with ED properties in the scoping study on pesticides conducted by Burger and Nel (2008) referred to earlier, notably Deltamethrin ($C_{22}H_{19}Br_2NO_3$) and Tralomethrin ($C_{22}H_{19}Br_4NO_3$), both of which are noted as having strong ED properties. Deltamethrin is listed as being used for the main crops cultivated in all of the 19 Water Management Areas detailed, and Tralomethrin in 16 of the areas, with both noted in the Inkomati area.

Although pesticides were not included as part of this study site observations as they are being addressed in a separate WRC project with some methodology still being developed (K5/1956), given the possibility for bromide exposer to be linked to pesticides as noted above, it may warrant inclusion for further studies. Verreault *et al.* (2004) noted that changes in thyroid hormone status in gulls presenting with elevated blood levels of halogenated organic contaminants [81].

It is noteworthy that the two phases of 2012 water and sediment samples collected for this separate WRC study from the Komatipoort site failed to produce any evidence of androgenic

or anti-androgenic activation or cytotoxicity (WRC Project K5/1956). However, the same samples yielded oestrogenic activity in the water and sediment media (0.136 ± 0.071 EEq ng/L for water, and 1.905 ± 1.295 EEq ng/kg for sediment). Cytotoxicity was observed in the water samples for the androgenic bioassays.

Since all these bioassays were conducted by the same specialists using the same protocol for both projects (K5/1915 & K5/1956) the variable results obtained highlight the variable nature of surface water quality and the need for monitoring and not simply once-off sampling.

One of the additional reasons for the variable responses observed between these projects relates to the variable presence of pesticides used within the sampling site area. The reader is referred to the scoping report by Burger and Nel (2008) on agricultural pesticides [82].

It is worthwhile noting that for broilers as sentinel models for EDC hazards for public health the obtaining of renal tissues (although potentially more insightful) is not always practical as the organs tend to be friable (poultry possess a combination of mammalian and reptilian nephrons with a unique portal blood supply). The deep pectoral and liver values do appear adequately reflective of the histopathology and blood hormone values to serve as valuable tissues for assessment.

Although this topic can be described in much greater detail this deliverable objective has been met inasmuch as endocrine disruption has been observed from both clinical, histopathological and production records which has been linked to differences in exposure via drinking water. The use of the same breed of broiler model, whilst possibly not essential, has to be viewed as preferable in terms of methodology.

In the consideration of the results obtained it is noteworthy that clear endocrine disruption was observed in both blood hormone values, endocrine gland pathology and supported by tissue observations. This is contrasted by the oestrogenic bioassays that failed to produce values that exceeded the trigger value of 0.7 ng/l for oestrogenic activity in drinking water [40].

It may subsequently be argued that in order for EDCs to be investigated, due to the uncertainties that exist regarding exposure to multiple EDCs with unknown or poorly described interactions, it is best to evaluate a combination of oestrogenic, androgenic and thyroid related responses.

The correct response to the observations made from the monitoring and assessment phases put forward in this deliverable for Set A sites would be to investigate Site 1 and Site 2 at the next level, namely affected communities. Continued use of a viable control site (such as Site 3) would remain a recommendation in order to assess seasonal variability.

What the methodology employed thus far does demonstrate is a differential focus for the sites in terms of cause (bromide for Site 2 and other potential pesticide sources for Site 1) but a similar initial phase in terms of investigating thyroid function in potentially affected communities.

In terms of human health the starting point of obtaining blood thyroid values for potentially affected and non-affected communities within the catchment would be a potential option.

It may be argued that given this variable response in the bioassays employed the use of longer exposure models, in the form of broilers as sentinel species, provides a valuable tool for evaluating EDC activity.

The use of sentinel species to determine EDC activity is described in more detail by Bornman *et al.* (2007) in the WRC Report No. 1505/1/07 [83]. Whilst a wider range of species were used in that study as biosentinels the use of a standard commercial breed of broiler has several advantages as previously noted.

In summary the methodology employed provides a repeatable means for investigating EDC exposures.

4.7 Case Study Set B: Kusile Power Station

4.7.1 Background

This site is characterized by the current construction phase of the Eskom Kusile Coal-fired Power Station (KPS) and was selected as it not only represents an area that has both naturally occurring and anthropogenic potentially hazardous chemical constituents in the form of recognized inorganic trace elements and coal-associated elements noted by the US EPA as EDCs, but also has sensitive receptors in close proximity in the form of wetlands and multiple water use types (domestic and agricultural).

As one of the largest construction sites in South Africa (planned completion date in 2018) it serves as a valuable monitoring site of potential hazardous chemicals that may have EDC effects associated with construction activities. The concurrent phasing in process of various units from 2014 will provide monitoring and assessment opportunities for EDCs associated with both construction and operational aspects for coal-fired power stations. Given the increase in the number of coal mines and smaller coal-fired power stations in South Africa this is viewed as a research priority in terms of EDC management.

Located in the Delmas Municipal area of the Mpumalanga Province KPS will be the 4th largest coal-fired power station in the world. Coal reserves are located all around the KPS site with active mining already present and the largest new proposed mine to supply KPS, the New Largo reserve, currently

underway with the Draft Scoping EIA (Anglo Coal South Africa). The KPS construction area is approximately 2500 ha with the whole site encompassing 4500 ha, but additional area has been applied for (1500 ha) to serve as a 60-year Ash Waste Disposal Facility.

In addition to the current construction phase of the power plant, future locations of coal-transport conveyor belts, future open-cast mining operations and locations of coal-ash dumps, all serve as potential sources of EDCs.

The potential exposure pathways for KPS are numerous involving air, surface runoff and subsurface migration to surface and subterranean water resources. The potential receptors are sensitive aquatic environments (wetlands associated with key rivers in the Upper Olifants Catchment), agricultural practices (crops and animal production), and human settlements. The close proximity to the KPS and planned Ash Dumps of a Pigs Genetics Facility (PGF) was an additional consideration in the site selection process enabling EDC aspects relevant to the reproductive and growth axis in pigs to be investigated.

The use of pigs as a model for predicting possible human effects is well-documented in the medical literature and the selected production unit is ideal as the PGF is a global pig breeding organization that specializes in the export of genetic animal material with breeding facilities located on the western boundary of the KPS. As part of a global partnership structure the PGF participates with 50 countries with genetics data, reproductive parameters, health status reports and semen quality reports captured on a standardised central data base every month. Thus, in addition to the high herd health status with strict biosecurity measures in place (a benefit for reducing disease as a confounding factor in EDC effects), a major consideration in the selection of the site related to this fact that the production records are collated both locally and internationally allowing for comparisons of reproductive, growth and performance parameters on a monthly basis to be made on an international level. The observational data set and end-points being monitored provide an ideal link to both the reproductive and thyroid pathways relevant to EDCs not just for South Africa but also with potential for international collaboration which could thus also involve analytical and bioassay comparisons.

The production unit has over ten years of production data with an artificial insemination section providing the opportunity to investigate a combination of fertility aspects ranging from litter size, embryological and neonatal developmental aspects, to semen quality. This provides a crucial core genotype based on application of genetic breeding data from a central European point with a variation reduction in subsequent genetic EDC-related component. This in effect allows for more confidence to be attributed to any observed EDC exposure- related effects.

The selection of the site is also preferred due to the physiology of the porcine which more closely resembles human inferences that other animal types (ruminants for example). As an example for use of pigs in studies for human health Peruzzi *et al.* (2010) proposed the use of a sentinel PIG-A gene,

which encodes one of the subunits of an enzyme essential in the biosynthesis of glycosylphosphatidylinositol (GPI), as a practical measure of mu (mutation rate) in human cells [84].

The KPS air, surface and groundwater monitoring is conducted via an Environmental Monitoring Committee (EMC) as per relevant DEA and DWA ROD, licences and authorizations. This provides the potential for an on-going monitoring data set for air and water quality that may serve as an input to the monitoring and assessment of EDCs for future EDC research projects and an opportunity to evaluate how effective current legalisation and environmental practitioners (companies, environmental control officers and environmental officers) incorporate EDCs in the related processes of EIAs and monitoring and assessment. Extensive environmental monitoring-related tasks were completed and linked to other WRC projects. Due to the volume of these tasks they are not presented here and the reader is referred to the Deliverable 6 report for further detail. This section only provides a brief overview of the key issues.

It should be noted that the investigations for this site were viewed as an attempt to establish a suitable monitoring platform with baseline conditions adequately described in order to allow for future operational impacts to be evaluated.

4.7.2 Brief Overview of Methods

The KPS monitoring activities include several years of monitoring data from a variety specialist studies for the Environmental Impact Assessments required for numerous licences and authorisations for the proposed activities. These include surface and groundwater and air (dust) that are part of a separate ever changing monitoring programme operated by KPS as part of the EMC process. Only critical aspects relevant to these observations in terms of monitoring and assessment are presented here.

Based on the initial results point of use samples were selected to represent the cumulative contributions of the dual borehole supplies relevant to the various sites. Some additional sample sites were selected based on surface water investigations conducted on that included additional surface water points around the KPS Catchment area. Although these results are not presented here they did highlight downstream concerns regarding KPS runoff and were also supportive of the KPS EMC groundwater and surface water observations that have led to the issuing of several Event Notices alerting affected parties of observed hazards and a more widespread Contaminant Study.

The samples collected from the PGF were:

Water Quality
o Full inorganic chemistry o Microbiological indicator organisms o Oestrogenic and Androgenic bio-assays o Physico-chemistry
• Surface water points included: o the receiving stream from KPS the Klipfontein Spruit o the Wilge River entry and exit o The Klipfontein Spruit and Wilge River both enter and join on the PGF property. • Groundwater points included: o 7 groundwater sources ▪ 4 of these are located within 50 m of the Klipfontein spruit and according to geohydrological assessment are influenced by the surface water and runoff from KPS. o 4 Points of Use ▪ These are also referred to as “Sites” and relate to separate production units on the property. Each of these units is operated under strict biosecurity measures as separate units. ▪ The majority of these sites receive dedicated water from specific groundwater sources. • Water samples for bioassays were collected from the surface water sampling points and the main site point of use (groundwater source).
Tissue Samples
o Fluoride investigation for chronic dental and skeletal fluorosis conducted by designated herd veterinarians.
Sediment samples
o For Oestrogenic and Androgenic bioassays o Sediment samples were collected from the surface water sampling points for the bioassays.

The following table provides a summary of the key KPS tasks addressed. Although not described in great detail here they do provide an indication of the extent to which similar aspects may be considered in other large-scale EDC investigations.

Summary of Key Set B KPS Tasks Completed

Task	Comment
Monitoring: Water Quality Surface and Groundwater March 2011 + July 2011 + August 2011 + March 2012	**Results provided an indication of**: Baseline Exposure for Different Sites Evidence of background EDCs Evidence of variable pollution incidents March 2012 results validated by Full Quantitative ICP-MS
Health assessment in Pigs: Clinical evaluation for chronic fluorosis as indicated by exposure hazard conducted by specialist veterinarians.	**Key Observations:*** No evidence of acute fluorosis. No evidence of significant chronic fluorosis over exposure period. Reproductive- and Growth-related EDC effects routinely monitored by specialist veterinarians.
EMC Meetings and Specialist Report Reviews: October 2011 + December 2011 + February 2012 + April 2012 + June 2012	**Critical Flaws, Errors and Data Gaps detected and reported.** Several meetings were held and Technical Documents exchanged with the General Manager of KPS and Directors at Eskom and appointed Environmental Consultants to resolve monitoring and assessment errors. Key issues were addressed with the current status that the required EDC list has been submitted, accepted and included by Eskom for monitoring purposes.
Relevant EIAs: Document reviews and Specialist Consultations	**Related Environmental Source and Pathway Assessments include**: KPS Ash Storage Facilities. Phola Overland Conveyor System. Anglo New Largo Colliery. Development of Water Quality Guidelines for Wilge River
DEA & DWA: Meetings and Consultations regarding EIA, ROD and WUL conditions	**Key comments**: The recognition of EDCs in environmental monitoring is severely lacking. Agreement has been reached regarding the relevance (source assessment) and detection limits required (receptor assessment).
*It is noteworthy that the recent review by the US EPA of the Drinking Water MCLG for Fluoride recommended it be lowered from the 4 mg/L to below 2 mg/L with the emergence of growing concern and evidence of endocrine effects (predominantly decreased thyroid activity and hyperparathyroidism) and sub-clinical exposures.	

4.7.3 Key Results

4.7.3.1 Inorganic Water Quality Results

Combined Key Inorganic chemistry values observed from several phases (mg/L).

Samples	Water Quality Constituent (mg/L)			
	Total Dissolved Solids	**Iron**	**Bromide**	**Anomalies**
Sources:	49	0.348	0.031	F = 0.06; 0.04; 8.55; 8.23; 1.79; 2.64; 7.72
Boreholes n = 7	51	0.53	0.021	As = 0.022; 0.006; 0.074
	179	0.542	0.091	Mn = 0.206; 0.445; 0.445; 0.012
	217	0.708	0.076	Se = 0.078
	140	2.809	0.027	Pb = 0.012
	125	0.719	0.033	Ti = 0.058
	367	0.632	0.360	Zn = 0.330; 0.276
	50	0.035	0.008	
Points of Use n = 3	125	1.263	0.044	F = 2.20; 2.4; 8.57; 11.02; 0.09; 0.05; 2.46; 11.2
	117	0.28	0.015	As = 0.025; 0.017; 0.074
	190	0.411	0.110	Cu = 0.176; 0.081
	225	0.003	0.060	Mn = 0.361 Hg = 0.008
	48	0.509	0.024	Se = 0.074; 0.058; 0.078
	42	0.32	0.002	Mo = 0.010; 0.115
	120	1.12	0	pH = 9.17
	236	2.01	0.010	W = 0.376
	53	0.023	0	Zn = 0.214
Wilge River – Upstream	330	0.56	0.14	SO_4 = 140 Mn = 0.110; 0.132
	237	0.54	0.012	F = 0.2
		0.184		As = 0.073 Al = 0.130
				Se =0.069
Wilge River – Downstream	146	0.58	0.082	Se = 0.008 Mn = 0.126
	157	0.88	0.074	Al = 0.114; 0.231
		0.385		
		0.553		
Klipfontein Spruit	99	6.69	0.016	Cr = 0.014 V = 0.015
	279	1.0	0.013	Ti = 0.361 Pb = 0.002
		0.959		F = 12.9 As = 0.074 Se = 0.076
				Mn = 0.298 SO4 = 168
				Al = 0.605
pH range [6.79-8.04]; [7.15-9.17]; [7.14-9.16]				
All nitrate concentrations <3 mg/L				

4.7.3.2 Physico-chemical results

Combined Key Physico-chemical values observed (mg/L).

Samples	Water Quality Parameter			
	Ammonia (as N mg/L)	**Chemical Oxygen Demand** (mg/L)	**Suspended Solids** (mg/L)	**Surfactants** (mg/L)
Source: Borehole: n = 2	<0.3; <0.3	<10; <10	24; <5	<10; <10
Points of Use: n = 3	<0.3; <0.3; <0.3 <0.3; <0.3; <0.3	<10; <10; 22 <10; <10; <10	<5; <5; <5 <5; 40; <5	<10; <10; <10 <10; <10; <10
Wilge River – Upstream	<0.5; <0.3	18; <10	11; 64	<10; <10
Wilge River – Downstream	<0.3; <0.3; <0.3:<0.3	47; <10; <10; <10	24; <5; 14; 63	12; <10; <10; <10
Klipfontein Spruit	<0.3; <0.3; <0.3	14; <10; <10	27; <5; 134	13; <10; <10
Reference TWQR	<0.5	6-9	<3	<10

4.7.3.3 Microbiological Results

Combined Key Microbiological values observed.

Samples	Water Quality Indicator Organisms		
	Total Bacteria Count (Count/mL)	**Coliform Bacteria Count** (Count/100 mL)	***E. coli*** (count/100 mL)
Sources: **Boreholes n = 7**	40; 55; 61; 450; 1950 33; 5900;	45; 78; 25; 1; >201 4; >201	1; 1; 0; 1; 201 0; 41
Points of Use n = 3	285; 16100; 57 **1400;** 18; 3; 30 175; 19; 95	43; 41; 74 **70;** 5; 0; 1 **36;** 0; 2	0; 0; 1 **1;** 0; 0; 0 **17;** 0; **2**
Wilge River – Upstream	640	**>2 419**	**613**
Wilge River – Downstream	260; 880; 2800	**>201; >2 419; >2 419**	**29; 613; 727**
Klipfontein Spruit	300; 4800	**>201; >2 419**	**201; 579**
TWQR	**0-100** Recommended Action Level = 5000	**0-5** Marginal Risk 10-10	**0**

4.7.4 Bioassay Results

4.7.4.1 MDA-kb2 Reporter Gene Bioassay

This assay was used to determine the androgen activity of water and sediment samples.

Table 35. RB7. Summary of Androgenic Bioassay responses elicited in Water samples for Set B.

Set B Samples	Activation of Androgen Receptor	Inhibition of Androgen Receptor (REP20)* µg F-eq/L	Cytotoxicity
Klipfontein Spruit	No	715.77 ± 1.66	No
Point of Use	No	No	No

* Relative effects potencies (REP 20-80) ratio between EC20-80 values of the reference control and samples submitted expressed as flutamide equivalents.

Table36. RB8. Summary of Androgenic Bio-assay responses elicited in Sediment samples for Set B.

Set B Samples	Activation of Androgen Receptor (REP20)* ng T-eq/g sediment	Inhibition of Androgen Receptor	Cytotoxicity
Klipfontein Spruit: Upstream	13.76 ± 0.76	No	No
Klipfontein Spruit: Abstraction	18.27 ± 2.93	No	No
Wilge River: Upstream	1.28 ± 0.41	No	No
Wilge River: Abstraction	0	No	No

* Relative effects potencies (REP 20-80) ratio between EC20-80 values of the reference control and samples submitted expressed as bio-assay derived equivalents.

4.7.4.2 Yeast Estrogen Screen (YES) and T47D-KBluc Reporter Gene Bioassays

These assays were used to determine the oestrogenic activity of water and sediment samples. Since some of the points of use utilise subterranean sourced water distributed to the production site no relevant sediment sample sites were applicable.

Table 37. RB9. Summary of the YES bioassay responses in Water samples for Set B.

Set B Samples	YES assay*	Cytotoxicity
Klipfontein Spruit	No oestrogenic activity detected	None reported
Point of Use	No oestrogenic activity detected	None reported

* Activity expressed as oestrodial equivalents interpolated from standard oestradial curve.

Table 38. RB10. Summary of the T47D-KBluc bioassay responses in Water samples for Set B.

Set B Samples	T47D-KBluc assay* EE(ng/L)	Cytotoxicity
Klipfontein Spruit	0.167 ± 0.06	None reported
Point of Use	0.008 ± 0.002	None reported

* Activity expressed as oestrodial equivalents interpolated from standard oestradial curve.

^ Note cytotoxicity can mask oestrogenic activity.

Table 39. RB11. Summary of the T47D-KBluc bioassay responses in Sediment samples for Set B.

Set B Samples	T47D-KBluc assay* EE(ng/L)	Cytotoxicity
Klipfontein Spruit	No oestrogenic activity detected	None reported
Point of Use	No oestrogenic activity detected	None reported

* Activity expressed as oestrodial equivalents interpolated from standard oestradial curve.

4.7.5 Tissue Results

Based on the water quality results for the inorganic chemistry demonstrating exposure to potentially hazardous fluoride concentrations the consultant veterinarians conducted an investigation into risk for chronic fluorosis.

The site chosen with the highest fluoride values was chosen. A total of 6 male pigs were identified and at slaughter tissues were obtained at the abattoir and a clinical investigation was conducted for signs of chronic fluorosis.

The urine values obtained presented with a median value of 4.1 mg/L (±0.568) with all samples within the normal ranges for pigs. No clinical signs of enamel hypoplasia were evident in the dentition evaluated. No gross signs of exotosis were observed either.

Despite the high exposure concentration, the lack of clinical signs for chronic fluorosis can be attributed to the mitigating effects of a high nutritional plane and controlled environmental housing conditions. These effectively reduce the total exposure dose and assist in renal clearance rates of ingested fluoride. It should be noted that adverse effects in livestock are typically observed in production systems with multiple exposure routes (e.g. pastures and geophagia).

Since the target water quality range for fluoride is based on the incidence of fluorosis it remains a point of monitoring to observe any potential EDC effects.

4.7.6 KPS Environmental Monitoring Committee Results

4.7.6.1 Overview

As part of the environmental authorisation and water use licence conditions the KPS holds Environmental Monitoring Committee Meetings (EMC) approximately every two months. It is at these meetings that compliance monitoring is reported on by numerous specialists appointed by KPS/Eskom to perform the various monitoring duties.

In addition the EMC meetings serve as an opportunity to identify areas of concern that may arise from the monitoring data, or gaps therein. Critically the EMC process provides a platform from which affected and interested parties may raise issues that relate to the operations and impacts of the KPS and related activities. The final outcome of each EMC meeting is to produce an EMC Report, which is compiled by the Chair of the EMC, and submitted to the designated relevant parties at the DEA and DWA.

This EMC Report is largely based on an ECO Report (Environmental Control Officer) that is presented at the EMC meeting which is in turn largely reliant on the specialist reports referred to earlier. The burden thus rests largely on the various specialists to conduct the monitoring and reporting activities appropriately.

As has been observed with the multidisciplinary approach required for EDC research, this is a similar scenario where multiple diverse disciplines are required to address the key environmental issues. Communication between these different specialist fields thus represents a similar challenge.

In terms of perspective it is appreciated that EDC monitoring is not yet a formal requirement. It may be argued that it is by implication a fundamental component due to the guiding principles of the relevant Acts, Government Notices, and conditions stipulated in authorisations and licences granted for the KPS activities.

The ability to engage with the relevant parties ranging from applicants to competent authorities, in terms of including EDCs for monitoring and assessment purposes where applicable and relevant, is viewed as a key issue that can benefit EDC research, awareness and ultimately management thereof in South Africa.

The key requirements are to:

- recognise that activities requiring authorisations and licences provide an opportunity to include EDC monitoring in the conditions stipulated

- recognise that the EDCs may be included during the Scoping phase of EIAs by specialists and affected parties, or by the competent authority issuing the authorization or licence.

- recognise that in order to obtain meaningful quality data for EDC purposes requires attention to be given to sampling and analytical detail, notably in terms of detection limits.

- recognise that the inclusion of EDCs for monitoring purposes allows for the proactive management thereof and accords with the precautionary approach adopted for EDCs both internationally and locally.

This topic is dealt with in great detail in the Deliverable 6 for K5/1915 and is not repeated here. As a summary statement it may be observed that the initial EMC monitoring processes did not adequately monitor potentially hazardous constituents related to coal-fired power stations and related activities, both in terms of elements selected and detection limits. This was true for surface and groundwater monitoring and air quality monitoring. This was also a general observation relevant to the numerous applications for other related activities in the area, for example, coal mining, transport and storage of coal. With the failure to recognise documented potentially hazardous constituents (both in terms of water quality and hazardous air pollutants) it followed that EDCs were not adequately addressed either.

After numerous engagements the vast majority of the inadequacies were addressed and the KPS EMC now represents possibly one of the best surface and groundwater quality monitoring data sets for EDC research. It should be appreciated that the current construction phase has allowed for a baseline data set to be compiled and future commencement of operations will present an ideal

opportunity to investigate impacts on a wide range of receptor types, from sensitive wetlands, sentinel models, to communities.

A brief summary highlighting the key lessons learnt is provided next with the reader referred to Deliverable 6 of WRC K5/1915 for further detail.

4.7.6.2 Key Lessons Learnt

Within the relevant legal framework it may also be noted that pollution control and waste management form an integral part of sustainable development, with a principle of using utmost caution when permission is granted for new developments. Environmental impact assessments form a critical aspect of the process.

Similar to a water use licence application, in terms of the relevant Acts applicable for environmental management and water, providing false or misleading information in these reports from the specialist, to the ECO and EMC Report, is a criminal offence.

This aspect warrants the adoption of the precautionary principle where monitoring and assessment aspects are concerned specifically where human health may be impacted on. This is particularly relevant for endocrine disruption effects as comprehensive descriptions of cause and effect relationships are difficult to describe.

The objectives of involvement in the EMC meetings were:

- To evaluate the ability of current monitoring and assessment processes as applied to the KPS to address the internationally published and recognized EDCs relating to coal-fired power stations, coal-mining, transport, storage and related activities.

- To establish a baseline model and to identify the gaps currently existing regarding the EDC issues within the catchment as relevant to KPS.

As an indication of the general nature of the monitoring and assessment concerns, the scientific literature generally reports potentially hazardous trace elements in the environment associated with coal-fired power stations to be:
Arsenic, Aluminium, Antimony, Barium, Beryllium, Bromide, Cobalt, Chromium, Copper, Iron, Lanthanum, Lead, Manganese, Mercury, Molybdenum, Nickel, Selenium, Silicon, Strontium, Tungsten, Uranium & Vanadium

Despite these all being able to be routinely sampled and analysed in water quality investigations it is noteworthy that in addition to this list relating to the source characterisation of KPS, 15 of these constituents also appear in the Standard Limits for SANS 241:2011 Part 1, yet only 3 were initially addressed in the EMC Meetings via the Surface and Groundwater monitoring reports.

Although the following sections highlight many of the difficulties and fundamental flaws that were initially observed it should be noted that these challenges can be expected in other similar monitoring and assessment study sites. The purpose of this section is thus to highlight some of the critical error and fundamental monitoring and assessment challenges that can be used to hopefully ensure better application thereof at other study sites.

As this section adopts the basic source, pathway and receptor approach it had to include related environmental draft scoping reports for proposed 60 year Ash Waste Disposal Facility, Coal-mining (New Large Colliery) and the proposed turbidity guidelines for the Wilge River.

As a summary statement, the process culminated in the following positive outcomes:

- The commencement in May 2012 by KPS of the monitoring of inorganic EDCs in surface and groundwater by using the same ICP-MS methods as detailed in WRC Report K8/999 using full quantitative techniques through Pelindaba Analytical Laboratories at NECSA.

- The initiation of a larger catchment study by KPS to identify the sources and pathways (and in some instances receptors) for the key potentially hazardous constituents observed.

- The inclusion of additional monitoring activities, notably for microbiological indicator organisms.

- The inclusion of air quality, as defined by composition of recognized hazardous air pollutants, and not only dust monitoring (particulate matter).

- An open invitation by the Sustainability and Innovation Department of Eskom for interested researchers to collaborate on these and other projects (not just confined to EDC aspects).

4.7.6.2.1 Air quality

A central concern noted in several Scoping and Monitoring reports relates to the lack of monitoring hazardous air pollutants (HAPs) from coal mining, transport, combustion and storage, thereby precluding baseline determination. Since international publications recognise the environmental and public health hazards of constituents relevant to these activities, many of which are recognised as EDCs, it is reasonable to include them in monitoring and assessment procedures.

It is published that concern for trace elements in combustion technology to be employed by KPS and subsequent FGD byproduct (e.g. arsenic, selenium and mercury) limits the utilization of FGD byproduct and that the release of FGD byproduct is a barrier impacting utilization thereof. It is reported in the literature that trace elements may be captured by fly ash and coarse ash with

consequent significant environmental concerns as many are reported to be carcinogenic, toxic and potential endocrine disruptors.

Studies note that disposal of the ash may be accompanied by dissolution of calcium that may lower the pH and calcium concentration in the leachate facilitating the release of arsenic and mercury, which may be argued to represent a greater environmental hazard.

The key carcinogenic elements most frequently cited include arsenic, cadmium, nickel and zinc, whilst toxicity concerns are most often reported for selenium and mercury. It should again be noted that many of these are noted as EDCs, with related effects relevant as the inhalation pathway is generally considered more hazardous due to the lack of protective hepatic bio-transformations as entry is gained via the pulmonary circulation into the systemic circulation.

The HAPs which are integral components of the particulate matter emitted from coal-fired power stations include:

- Arsenic
- Beryllium
- Cadmium
- Chromium
- Lead
- Manganese
- Mercury
- Nickel
- Radium
- Selenium

These elements are reported in the scientific literature to affect toxicological, carcinogenic and endocrine disruptive routes. There are numerous peer-reviewed scientific journals in which the inorganic constituent hazards are linked to the potential source activities relevant. A report presented in March 2011 on Emissions of Hazardous Air Pollutants by Coal-fired Power Plants to the American Lung Association refers to the National Emissions Inventory prepared by the United States Environmental Protection Agency (USEPA) indicated that emissions to the atmosphere by coal-fired power plants contain 84 of the 187 HAPs identified by the USEPA as posing a threat to human health and the environment. A separate USEPA study (in 2007) also notes that the single largest point source category for mercury and arsenic releases into the atmosphere occur from coal-fired power plants.

It follows that monitoring these HAPs should be a requirement for air quality monitoring programmes, yet many of the Scoping reports present only estimated calculations for air quality hazards based on a single study conducted in November 2003 for Kendal Power Station where coal, coarse ash and fly ash samples were analysed. It was acknowledged that the "quantification of trace metal releases was restricted to those studied and documented in the November 2003 study. Furthermore, data were unavailable to quantify gaseous trace metal releases…".

As a general conclusion most reports thus failed to include inorganic hazardous air pollutant (HAP) testing despite these analytical techniques being available. Since no baseline conditions were established at the KPS site, or for the existing Kendal site, regarding inorganic elements recognized as HAPs associated with coal-fired power stations, subsequent statements on hazards and risks presented are inherently flawed, with potential risks purely speculative and based on a single study conducted in 2003.

It is relevant to note that these reports do in places acknowledge some of the hazards, one highlighting 28 trace elements considered relevant internationally with 22 trace elements are recorded as being detected as heavy metal releases from stacks, coal and ash dump operations.

Some notable omissions were evident, for example, fluoride/fluorine. Fluoride is reported in the international scientific literature as a priority pollutant associated with coal-fired power plants.

The EMC air quality monitoring reports present data sampled weekly from pre-selected sites for dust monitoring only, reported in mg/m^2/day with the action level set at 1200 mg/m^2/day. Measuring dust fallout precludes any meaningful statement on exposure to HAPs as no source, pathway or subsequent receptor exposure hazards can be calculated. As no baseline data is being gathered impacts to the surrounding environment cannot be observed, with trends in current and potential future increases in those HAPs specifically associated with the source characterisation relevant to the KPS and related activities remaining undetected.

These critical data gaps continued to persist in the KPS EMC Monitoring reports in 2013, with only a recent indication by KPS/Eskom that some composition in terms of air quality/dust fallout for specific constituents (pollutants and hazardous substances) will be monitored to establish current air quality.

Air Quality Summary:

The sources, pathway and receptor approach is fundamental to the assessment of hazards and risks and accepted world-wide, and implied in the relevant NEMA and NWA Acts.

Observation in terms of sampling, analytical determination and transparent reporting, of the relevant potentially hazardous constituents should be included for all these aspects (waste stream and other possible sources; pathways as relevant, e.g. air, soil, water, plant; for relevant receptor types).

Dust monitoring reports do not provide any meaningful monitoring or assessment data, preventing the establishment of baseline conditions for potentially hazardous constituents to public health and the environment relating to coal-fired power stations, mining, transport and storage of coal-combustion products.

The relevance of the air exposure route to the water-focus of this report is that in order to establish not only risk, but risk management strategies, it is critical to have information on all routes of exposure. This allows for perspective to be gained regarding the priority route for a specific receptor type (e.g. crops or drinking water). Thus, it is required to comprehensively sample and analytically determine those constituents in order to assess risks associated with the inhalation pathway for HAPs and EDCs.

4.7.6.2.2 Surface and Groundwater Quality

Initial surface and groundwater monitoring reports submitted and presented in the EMC meetings contained numerous incorrect statements that not only failed to accurately assess the potential impacts of KPS, but were also misleading regarding the data obtained from the monitoring processes.

The key issues were finally resolved and serve as a valuable lesson in the difficulties of implementing monitoring and assessment programmes for large scale Category B activities. These activities relate to the National Environmental Management: Waste Act (59/2008) GN 718 (of 9 July 2009) and refer to activities that have high risk in terms of environmental impact and which present effects that are not well described or easily predicted, and thus require an EIA to be performed.

This topic is dealt with in substantial detail in Deliverable 6 for WRC K5/1915 with only the key issues briefly highlighted here.

Microbiology

Initially no microbiological quality monitoring reports were presented at the EMC meetings. However, following concerns regarding environmental incidents reported at the EMC meetings of septic tank overflows and problems relating to the handling of the daily effluent load on the site from construction personnel it was requested that water quality tests be performed for microbiological indicator organisms.

Subsequently standard microbiological indicator organism tests were implemented in tandem with other water quality monitoring conducted on surface and groundwater points. This is viewed as relevant to the EDC context as these results revealed a widespread repetitive observation of significantly elevated Total Coliform and *E. coli* counts classified as completely unfit for use for domestic, animal watering and irrigation purposes. This not only resulted in several Incident Notices to be sent out to Affected Parties, but in a larger Contaminant Study being initiated. The extent of the significant infectious disease transmission risks presented by the monthly surface and groundwater

monitoring highlight the difficulty in relating adverse effects to EDCs when background risks from established water quality norms are already present.

The outcome of site-specific human and animal health risk assessments are thus influenced by general water quality and not only EDC exposures, and accordingly need to be established in order to prioritise and place context on potential EDC-related impacts.

It should be noted that whilst the microbiological indicator organisms are not in themselves EDCs they also provide an indication of other potential pollutants linked to runoff from the KPS construction site with similar environmental incidents applicable and thus serve as an indication of potential pollutant impacts. Since the source characterisation (noted for the industry and activities involved) presents with a high number of potential EDCs this aspect is viewed as relevant to the monitoring and assessment approach.

An additional issue emerged regarding the interpretation of the high coliform counts during the source characterisation. At some EMC meetings it was claimed that the *E. coli* to Total Coliforms ratio could be used to determine if the coliform counts were from human or animal origin. This claim is rejected as outdated and actually of reference to comparisons between faecal coliforms and faecal streptococci which has subsequently been unequivocally demonstrated as invalid and are no longer accepted and noted as such in the scientific literature, with the US EPA noting this as having been used in the past but no longer recommended as a reliable indicator of the origin of faecal waste. This highlights the need to remain updated with the developments in monitoring and assessment methods, from sample collection to the interpretation of analytical results. This point is valid within the EDC context as so much new information is continually being published.

Inorganic EDCs

Although many issues were relevant to this topic, the last point noted above regarding methods was illustrated by the initial EMC meetings with water quality monitoring data not including constituents noted as being pollutants and EDCs associated with coal fire power stations and related activities during construction and operational phases. These were also not sampled correctly, notably for trace elements including the EDCs arsenic and selenium, with the detection limits employed initially above the upper limits as per local and international water quality guidelines. This not only precluded a statement on fitness for use being made, but with EDCs requiring lower detection limits also limited baseline sensitivity required for EDCs.

The scientific literature generally reports potentially hazardous trace elements in the environment associated with coal-fired power stations to be:
Arsenic, Aluminium, Antimony, Barium, Beryllium, Bromide, Cobalt, Chromium, Copper, Iron, Lanthanum, Lead, Manganese, Mercury, Molybdenum, Nickel, Selenium, Silicon, Strontium, Tungsten, Uranium & Vanadium

The initial detection limits for Selenium and Arsenic values are all reported as ≤0.1 mg/L**.** This is insufficient from which to claim compliance or accuracy as the required detection limit is in the 0.001 mg/L to 0.01 mg/L range as the SANS 241:2011 Standard limit for both these constituents is ≤0.01 mg/L.

Some additional monitoring concerns were also apparent. It is noticeable that the EMC Reports did not allow for trending or sensitivity at the 10-1 µ/L range. Despite the already flawed data collected it was recommended at an EMC meeting that if 6 months of ICP-MS results showed "low" values then testing would cease.

This approach was rejected for three reasons:

- The point of monitoring is to establish baseline data over seasons – this would not be done if the recommendation was adopted.
- The monitoring is the only defence the affected parties have to show future pollution of potential hazardous wastes from the coal-fired power station and related activities. Ceasing with monitoring because the values are low removes the ability to observe pollution during further construction and operational phases.
- The current samples were incorrectly sampled and the analytical procedures employed insufficient to enable any trending to be done.

Summary

The key issues were subsequently addressed with samples collected in correctly acidified laboratory prepared sample containers and submitted to the appropriate laboratories for full quantitative ICP-MS procedures.

Proposed Turbidity guidelines for the Wilge river

Background:

As a major river within the catchment concerns were noted from the EMC meetings that the turbidity values were in almost all monitoring points elevated above the customised standard limit with some NTU values in the thousands. Photographic evidence was presented indicating the contribution from construction site runoff with Eskom/KPS subsequently formulating Proposed Turbidity guidelines for the Wilge river. This topic is viewed as an indication of the degree to which established water quality parameters may be addressed within a sensitive and already impacted upon catchment and thus as an indication of how EDCs may potentially be accommodated.

It should be noted that by implication the Water Use Licence does impose turbidity guidelines. The following WUL conditions refer:

> "Stormwater leaving the licensee's premises must in no way be contaminated by any substance, whether such substance is a solid, liquid, vapour or gas or a combination thereof which is produced, used, stored, dumped or spilled on the premises."

> "Pollution caused by the infrastructure impeding and/or diverting flow of watercourses as well as alterations to watercourses on the property/ies must be prevented through proper maintenance and effective protective measures, or otherwise minimized and remedied."

> "Activities that lead to elevated levels of turbidity of any watercourse must be minimized."

It follows that in order to comply with these conditions of the WUL compliance is required for stormwater management. Guidance in this regard may be obtained from the DWA schedules for Prohibited Discharges for stormwater and the DWAF South African Water Quality Guidelines (1996). These guidelines address both turbidity and suspended solids and related water quality constituents and may also be used to ensure that the quality of the water used by recognized water uses is not adversely affected by the actions involved. This is of relevance to other sites and not just the KPS, specifically where industrial or domestic stormwater runoff is involved, as they present a high risk of containing organic and other hazardous pollutants.

Proposed Upper Limit

The turbidity limit proposed was the EPA Upper Numerical Limit of 280 NTU stated as valid by the specialist as "Eskom chose the EPA because it is the biggest environmental regulator in the world." This approach is not necessarily valid in all contexts, and locally derived limits are not only permitted, but advocated by many international organisations (e.g. WHO Drinking Water Standards). Limits chosen should be scientifically defensible and accord with South African water quality guidelines and conditions. As is noted in the DWAF (1996) South African Water Quality Guidelines for Aquatic Ecosystems the guidelines need to be considered on a site-specific basis.

In this instance it is critical to note that the 280 NTU limit proposed for the Wilge River actually refers to compliance of stormwater samples and not river or surface water samples.

The actual regulation is stated by the US EPA as:

> "All construction sites required to obtain permit coverage must implement a range of erosion and sediment controls and pollution prevention measures. These sites must sample stormwater discharges and comply with a numeric limitation for turbidity. The limitation is 280 NTU (nephelometric turbidity units)."

Local and international turbidity limits are far more conservative, being in the 25-50 NTU range with 10-20% changes from background values permitted. Given that Turbidity is used as an indicator of

numerous other potential risks it follows that the relevant constituents affected should also be monitored. This approach is also noted in international water quality criteria/guidelines/standards.

The Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC, 2000) provides trigger values for different types of ecosystems, with more conservative limits applicable to the upland rivers which are arguably relevant to the KPS Highveld scenario (lowland river NTU Trigger Value 6-50; upland river 2-25) [85]

The proposed turbidity guidelines also intended to use turbidity as the single water quality parameter for monitoring the impacts of KPS on the Wilge river, with it being indicated that the correlations with turbidity and other water quality constituents being sufficient. This was fundamentally rejected as turbidity is not used in isolation in local or international guidelines. The attraction for reducing a monitoring programme to a single constituent, or a few constituents, is motivated for by cost, with a potential benefit of an increased frequency of monitoring thus possible. However, the consequent loss of valuable constituent information is significant and would prevent meaningful monitoring and assessments from being performed.

It was thus proposed that given the ability for stormwater runoff to contain numerous hazardous waste pollutants, the stipulations regarding stormwater be applied as detailed below with the Special Limit for Prohibited Discharge for Stormwater applied to Stormwater Discharge samples as published by the DWA:

Prohibited discharges into stormwater drainage systems.

PARAMETER	Special Limit
Faecal Coliforms(counts per 100 ml)	0
Chemical Oxygen Demand(mg/l) 30*	30*
pH	5.5-7.5
Ammonia (ionised and un-ionised) as Nitrogen(mg/l)	2
Nitrate/Nitrite as Nitrogen (mg/l)	1.5
Chlorine as Free Chlorine (mg/l)	0
Suspended Solids(mg/l)	10
Electrical Conductivity(mS/m)	50 mS/m above background receiving water, to a maximum of 100 mS/m
Ortho-Phosphate as phosphorous (mg/l)	1 (median) and 2.5 (maximum)
Fluoride (mg/l)	1
Soap, oil or grease (mg/l)	0
Dissolved Arsenic (mg/l)	0.01
Dissolved Cadmium (mg/l)	0.001
Dissolved Chromium (VI) (mg/l)	0.02
Dissolved Copper (mg/l)	0.002
Dissolved Cyanide (mg/l)	0.01
Dissolved Iron (mg/l)	0.3
Dissolved Lead (mg/l)	0.006

PARAMETER	Special Limit
Dissolved Manganese (mg/l)	0.1
Mercury and its compounds (mg/l)	0.001
Dissolved Selenium (mg/l)	0.02
Dissolved Zinc (mg/l)	0.04
Boron (mg/l)	0.5

* = after removal of algae

The presence of multiple recognized water use types from domestic use, agriculture to aquatic ecosystems require the most sensitive user type to be considered. Given the above, it was recommended that the <50 NTU applications of both local and international limits be used, effectively implying that:

- Background values may not increase more than 5 NTU at any one time
  - the default turbidity trigger value used for these surface waters should be 25 NTU implying that the upper limit should be set to within the 0-25 NTU range

Summary

Differentiation is required between source types, for example, discharge samples, streams flowing from KPS property into the rivers, and upstream and downstream monitoring, in order to allow for a meaningful pro-active approach. Fundamentally the use of a single water quality constituent for monitoring purposes as proposed (in this case Turbidity) is flawed and hence not recommended and particularly relevant in the case of an indirect measurement of potential hazards such as turbidity. The use of discharge limits for relevant parameters and specific use-specific water quality constituents presented in the DWAF (1996) South African Water Quality Guidelines is thus more valid.

The exercise for developing Turbidity guidelines for the Wilge river highlighted some of the complexities that will also be applicable to the management of EDCs in catchments and serves as an indication of the requirement to consider source, pathway and receptor aspects with the assistance of both local or site-specific observations, relevant guidelines and standards and internationally published reference works.

4.7.7 Discussion Set B

Inorganic Water Quality
The main concerns from the PGF monitoring observations relate to: Potential for chronic dental and skeletal fluorosis (enamel hypoplasia & skeletal exostosis & structural changes). Health investigation revealed no gross lesions or evidence of chronic fluorosis and exposure period and group appear thus within acceptable risk limits. Possible endocrine disruption risk related to both As, Br, Se and F. These should form the fundamental basis for any further EDC-related research with the pig model used for sentinel purposes. As the water quality monitoring results indicate these appear to be linked to highly variable upstream discharges and are not inherently elevated in the natural surface water environment. Some subterranean points may be influence by natural coal deposits that may contain these elements. Naturally occurring geochemical anomalies are therefore relevant in the associated environment and should be established during any investigation into potential total EDC exposures. Some adverse palatability responses due to predominantly elevated iron concentrations also observed. Elevated iron in line with reported mining contaminants located in the catchment. The elevated values may be linked to: - coal deposits. - coal combustion products and related activities - naturally occurring geochemical anomalies. - some decant pollution incidents. The mining activities as a potential source are also documented in EMC water quality reports. It was noteworthy that other potential inorganic EDCs (Cd, Cr, Se, Hg & V) present current baseline values below the TWQR for most of the observation points. The main conclusions are thus that: The current exposures reveal some persistent EDCs but critical pollutants associated with the expected source characterisation are elevated in intermittent surface water monitoring. This would suggest that the monitoring with the pig as a sentinel model should commence within a collaborative partnership between the industry and affected parties. Appropriate sampling, analytical procedures and assessment demonstrated by the PGF monitoring highlights the need for the current pre-operational and ash waste disposal activities to be monitored prior to the expected increased source and pathway exposures. Accordingly a more frequent monitoring programme is indicated and should be maintained that should include those constituents that can be reasonably expected to be of relevance to the source involved (coal fired power station and related activities). Correct methodology is essential to observe correctly the current situation and be able to formulate appropriate mitigation and risk management actions.

Physico-chemical Quality

General:

The Klipfontein spruit and the confluence sample share similar Suspended Solids and Surfactants results, both being significantly better for the Wilge River and potentially linked to pollutant runoff from construction activities from KPS and related activities (noted by numerous monthly environmental incidents).

The general Suspended Solid standard for discharge of effluents of 25 mg/L was being exceeded or almost exceeded by both the Klipfontien spruit and confluence samples. The higher COD for the Klipfontein spruit is also noteworthy. The value falls sufficiently outside the Target Water Quality Range to potentially cause the growth of microbiological slimes enhancing microbial growth of microbial-influenced corrosion. The observed value is still lower than the effluent discharge standard of 75 but a stricter discharge may apply for the specific receiving water quality objectives of the natural water course.

Overall Conclusion:

The Klipfontein spruit and Wilge river differ in quality with the Klipfontein spruit being worse and for some parameters near the upper limit for effluent discharge and supposedly linked to pollutants from KPS and related activities (refer to KPS EMC data).

The key boreholes on the Klipfontein spruit appear to be influenced by the surface water quality on the basis of both the analytical results and a site-specific investigation by an independent geohydrologist appointed by Eskom. The high values observed were supportive of the significant microbiological indicator organism risks observed. Upstream sources may extend to beyond the KPS but are currently subject to a wider catchment study by Eskom/KPS.

Bioassay Results

General:

The Androgenic responses observed indicated activation for the sediment samples and inhibition for one water sample. Activation was noted with greater potency for the Klipfontein spruit sediment samples but as is detailed in the bioassay report the observation of activation of the androgen receptor in sediment samples from both the Klipfontein spruit and Wilge river accord with the expectation of sediment as being a reservoir for EDCs.

The failure to observe inhibition in water samples at the point of use for the Klipfontein spruit may be due to a buffer effect since the point of use represents abstracted subterranean water that although influenced by the river quality (independent Eskom geohydrological reports) may not reflect yet in the water abstracted. The migration cone may either take some time for the EDCs to impact on the resource, or present with seasonal variation. Alternatively, as the sediment sample results suggest, the EDCs may be retained in the sediment to a sufficient extent that the subterranean quality does not get significantly impacted on.

The detection of oestrogenic activity in the water samples for both the source (Klipfontein spruit) and the point of use, although below the trigger value of 0.7 ng/L, further supports the concerns for the impact of coal mining and related activities in the catchment.

Only ongoing monitoring will reveal insight to these issues and as noted in the bioassay report the next step would also be to perform targeted chemical analyses to identify the compounds responsible for the bioassay results obtained.

In addition to those substances noted to be of relevance to the Eskom/KPS activities the potential for other mining and

Bioassay Results

construction related compounds may be relevant within the catchment. This may be partially addressed by the current Eskom/KPS Contaminant study being conducted. If this study reveals links to the other PHCs noted in the water quality results insight may be gained towards other potential EDCs.
A screen for pesticides should be included in such a study to differentiate between agricultural chemical contribution and that from mining-related activities.

Overall Conclusion:
The Klipfontein spruit and Wilge river provide an inflow into the Upper Olifants Catchment, the subject of separate CSIR Studies. These studies have observed histopathological results in fish thyroid tissues indicative of endocrine disruption. Although this study is yet to be finalised the potential links between the source, pathway and receptors involved should be investigated in a collaborative manner. The study has also observed that the poor quality of the Klipfontein spruit impacts adversely on the Wilge river with this effect having been studied to the confluence point. Improving the Klipfontein spruit water quality is viewed as integral to the subsequent adverse effects (SASS scores) on the Olifants river and related catchment.

To this end the researchers involved of both the Eskom/KPS Contaminant Study and the relevant CSIR studies for the Upper Olifants, have been notified and collaborative research projects are recommended.

In summary sufficient data has been collected for the Set B study site to motivate for additional EDC investigations with the current monitoring of the porcine reproductive and growth indicators in addition to the environmental samples already being monitored by Eskom/KPS a strong recommendation.

Microbiological Quality

General:
The *E. coli* and other indicator organism values observed are potentially linked to excessively elevated values reported in KPS EMC monitoring data for both surface and groundwater since commencement of monitoring thereof. Challenges in handling effluent from construction sites may be a common theme worthy of attention in similar investigations.

Conclusions:
Due to the observation of completely unacceptable quality and significant acute public health hazards the monitoring has to be accompanied by accurate statements regarding hazards and risk presented. These hazards must be communicated to affected parties and within an appropriate time-frame. Sections in Volume 5 of this Manual referring to Risk Communication refer.

The catchment study currently initiated should assist greatly in identifying and addressing the high hazards observed.

Handling of effluent generated by construction teams is not confined to the KPS but extends to the construction of over-land conveyor systems, the New Largo Colliery and other activities. This ability to handle the impact of human effluent may be viewed as relevant to other pollutants from these activities as they represent the ability of the impact studies and subsequent authorisations and compliance conditions to effectively protect the users within the catchment.

Air Quality
General: The lack of any composition description precludes any defensible statement from being made concerning baseline conditions and detecting exposure to HAPs and EDCs. As is noted in the US EPA guidelines on water resource protection the appropriate considerations for protection, management and prevention should be conducted on a case by case basis with careful consideration of the risks posed by the contaminants involved (http://water.epa.gov/polwaste/). In the American Lung Association's report on hazardous air pollutants and coal-fired power stations it was noted that in addition to the regulatory authorities involvement to address the hazardous mix of air pollutants, the involvement of associations dealing with public health issues, in this case the Lung Association, was required as well (http://www.lung.org/assets/documents/healthy-air/toxic-air-report.pdf). Conclusions: Appropriate studies (apparently to be commenced) must be conducted to address the lack of air quality and HAPs and EDCs relevant to the source characterisation. This should happen before operations commence as stockpiling of coal and use of fly-ash and related byproducts commences during the construction phase. These may also be relevant based on current related activities in the catchment. The source, pathway and exposure assessment should be conducted using the pig sentinel approach to establish as much of a baseline assessment as is currently possible.

Tissue Results
As the incidence of chronic fluorosis is a function of period of exposure, duration of exposure and seasonal effects the time and history of the pigs observed were instrumental in the findings. Specifically, the pigs were not exposed during the neonatal period and thus should not present with clear enamel hypoplasia. This is noted with ruminant stock that are exposed in feedlot situations to high fluoride levels (>10 mg/L) without incurring adverse effects due to the chronic cumulative nature of fluorosis. In the feedlot scenario the permanent teeth have already erupted and exposure duration is limited to approximately 100 days. The sampling period for these pigs was at the end of winter and water intake levels can be expected to be far lower. The molar dentition was also not inspected and may present with higher erosion and the incisors. Accordingly it was recommended to sample urine again at a 6 month interval and include routine observations at the abattoir for gross macroscopic lesions. In addition the testing of bone for fluoride values was recommended. Finally, as noted previously in this report in a recent US EPA Scientific Review of the EPAs Standards for Fluoride in Drinking Water (US EPA, 2006) it is noteworthy that not only was the upper limit for fluoride in drinking water (maximum contaminant level) reduced significantly but that some primary reasons noted for this were: ▪ "there are some data to suggest that fluoride does adversely affect some endocrine glands" ▪ "recent work on borderline hormone imbalances and endocrine-disrupting chemicals indicated that adverse health effects, or increased risk of developing adverse health effects, might be associated with seemingly mild imbalances or perturbations in hormone concentrations" ▪ "decreased thyroid function, secondary hyperparathyroidism, impaired glucose tolerance and possibly effects on timing of sexual maturity …may be associated with fluoride intakes that is

Tissue Results
achievable at fluoride concentrations in drinking water at 4 mg/L or less..."
It was thus also noted that whilst pigs are regarded as being tolerant to high fluoride exposures when compared to ruminants the endocrine disrupting effects have not been investigated. As with many EDCs the effects may be quite different for specific constituents when compared to the classic routes of toxicity that have been generally documented. Subsequently, the monitoring of thyroid values in blood was included as an additional recommendation for the exposed sites.
The potential for subterranean water quality to be influenced by the coal deposits noted in the area requires that these exposure sources be quantified prior to linking EDC effects to coal-mining and related activities. This accords with the site-specific baseline approach referred to in this Volume.

4.8 Water Quality Guidelines used for the Case Studies

It is important to note that for the models used (poultry and pigs) it is not a simple task to use the existing water quality guidelines for assessing the fitness for use. One of the primary reasons is that in terms of constituents listed and ranges considered relevant, the 1996 DWAF guidelines are outdated. In the case of the ROSS 308 broiler breed and strain specific constituents are supplied from the country of origin (United Kingdom) as part of a management guide. These are insufficient however as they fail to account for locally occurring WQCs and EDCs.

It is not uncommon to see animal producers use the SANS 241: 2011 Standard to assess water quality for animal use. The assumption is that if the water is good, or fit for use, for humans then it should also be good for pigs. This is incorrect for several reasons and would incur the same error as assuming it would be good for any other different purpose, such as irrigation or industrial applications (for which far stricter limits may apply).

The SANS 241 Standard is specifically designed with bulk water suppliers in mind and aimed at safe water provision for domestic drinking use and thus focusses on life-long safety for all types of users (infants, pregnancy, breast-feeding and adults) and aesthetic acceptability.

These aspects are not necessarily relevant to intensive animal production systems where variable exposure periods and requirements to meet genetic potential under the production conditions require a very different emphasis.

By way of comparison it may be observed that whilst the SANS 241 Standard provides a statement on safe drinking water it is also not intended to provide appropriate fluid replacement for people engaging in athletic activities.

The DWAF guidelines for livestock watering, and the SANS 241 Standard, do not provide appropriate current guidelines given the failure to accord with the trends noted for domestic use above.

As was noted in Section 3 of this report many South African animal production systems rely on subterranean water with a comprehensive constituent assessment required that may differ from the lists presented in international literature due to the influences of local geochemistry.

As guideline derivation generally relies on detection it stands to reason that many EDCs that are not routinely determined tend to be absent from guideline lists. Constituents relevant to their particular set of local circumstances, as noted for Set B case studies and by the variance in exposure values between Set A sites, should thus be included as opposed to omitted on the basis that they may not be reflected in regulatory guideline tables. Where such constituents thus fail to have either clear guideline values, or the guideline values relate to other types of effects (e.g. nitrate for methemoglobinemia and not EDC effects), the combination of detection limits, bioassay screening, and sentinel approaches as detailed in this section provide a viable means of obtaining sufficient information with which to make an informed decision. It still remains necessary to determine the fitness for use for the established norms in order to place any EDC hazards in context with other risks due to non-EDC exposures or types of effects.

The current DWAF (1996) guidelines for livestock watering were thus used although they provide for general classes of animals, such as ruminants and monogastrics, whilst subsequent research for the WRC has produced hazard and risk assessment methodology for more specific categories of animal production, including commercial, rural communal and wildlife enterprises. The reader is referred to the WRC reports for further reference detail (WRC Reports 857/1/01 & 1175/1/06).

As a brief indication to the norms and types of effects relevant for determining the fitness for use of water for the intensive animal production systems applicable to Set A and Set B the following may be considered relevant:

- Health: Toxicology
  Palatability
  Induced Deficiencies
  Disruption / Disturbances
- Product Quality: Product characteristics:
  Fitness for use for consumption
- Production System: Water system effects
  Application of water treatments
- Environment: Effects of animal and production system

The purpose is to assess fitness for use and to establish a monitoring platform. Consequently many WQCs are included that do not have specific guidelines for the specific user. But since it may reasonably be assumed that they may be relevant as more research information becomes available in the future, or that evidence of suspected endocrine disrupting activity exists (the case for the vast majority of organic WQCs), a precautionary principle is applied.

Fitness for use for animal watering is assessed using the generic guideline application approach as detailed in the WRC Final Reports 644/1/98, 644/2/98, 644/3/98 and 857/1/01, 857/2/01 and 1175/1/06. This approach is concentration-based, as opposed to ingestion based, and is therefore conservative in risk estimation. A more accurate risk assessment may be performed when intake of a constituent is known from feed, water and other sources, and combined with site-specific risk factors that may increase, or decrease, the concentration at which a constituent in the water source may have an adverse effect. Use of specific information in this manner forms part of an ingestion-based specific guideline application level and hence involves site investigations with various data capturing procedures.

Two aspects must be noted. Firstly, elevated water intakes due to osmo-sodium responses, high ambient temperatures, and palatability responses, significantly increase the risk for toxicity at any given guideline value (most are based on an average temp of ˚ C). Secondly, combinations of hazardous constituents in the same water source can be synergistic, or antagonistic, and as such, end effects may mitigate, or exacerbate, toxicity. Few water quality guidelines comment on recommended levels of water and diet/feed/forage levels simultaneously. Dual incorporation of these factors are at times impractical, for example, high variability between breeds and species make the incorporation thereof into guidelines difficult.

Finally, a generic level risk assessment is recommended as the first step in determining baseline exposures required for the identification of constituents in the geochemical environment that may contribute to adverse effects on health, productivity, and product quality. Any potential hazards identified would then require further investigation regarding the water, user, environment and nutrition.

These aspects are relevant for EDC investigations as they highlight the difficulties in applying assessments for those constituents that are supposedly already “well described”. It may be observed that EDC assessments are thus understandably more complex, and may thus require a more comprehensive monitoring programme to assist with these assessments.

4.9 Motivation for Including Biological Tissues

The use of biological tissues assists in identifying the relevance of baseline conditions and EDC effects. A primary reason for this relates to the concurrent exposure to multiple EDCs and other potentially hazardous constituents (e.g. organic). This may alter the pathway relevant from a dynamic and kinetic aspect with final outcomes ranging from synergistic to antagonistic. Thus, whilst

numerous clinical biochemistry applications exist for specific inorganic water quality constituents they primarily address significant deficiencies or toxicities. In some instances a great deal of information exists on a specific breed or species (as is typical with commercial livestock and companion animals) and more confidence in the reference values allows for more discriminatory interpretation of single constituent concentrations in individual types. This may extend to the confidence in bands of marginality, as in marginally deficient, or marginally excessive, as opposed to significant deficiency or toxicity.

This is however seldom the case and certainly not typical of EDC effects. For EDC effects the typical route is not a classical toxicity due to excess or clear clinical symptom of deficiency but rather a "disturbance" or, as is the term typically applied to EDCs, "disruption". Inorganic water quality constituents may result in endocrine disruption not only by primary direct effects, but due to induced deficiencies, for example, iodine-deficiency disorders. In this instance the disruptive effect is best thought of as an induced deficiency with a final effect to increase the requirement for an inorganic element required for specific endocrine processes. An example of this may be selenium requirements for iodine activation in thyroid function.

Due to these aspects the use of a suite of tissue types is preferred as opposed to single tissue values as this allows for trends between tissue types to be investigated within marginal bands.

It is thus recommended to perform the same ICP-MS techniques (following an acid digestion and/or drying) on more than one tissue type as opposed to only submitting one tissue type for a targeted full quantitative analysis of a single suspected inorganic constituent (e.g. liver sample for copper analysis). A similar approach may be valid for establishing other constituent concentrations in biological tissues, for example, residues for pesticides and related breakdown products.

It is also preferable to obtain sufficient sample numbers (individual animal observations) for adequate statistical interpretations of the analytical results. In this regard a biostatistician should be consulted in order to obtain the correct number of samples required. It is also strongly recommended to consult a specialist (animal scientist or veterinary scientist) as the interpretations of the analytical results may require different reference sample sites (for example specific hepatic lobes) to be obtained that vary between animal species.

Generally, the sample acquisition may coincide with other supportive information gathering objectives such as histopathology. In this regard a pathologist may assist with the number and specific tissue required for the species being used.

Different tissue types may also be analysed separately or pooled to acquire sufficient sample size. It is thus necessary to consult with the laboratory in order to obtain the acceptable sample mass for the analytical procedure required.

Some preparation may also be required regarding grinding and fat removal (e.g. bones) and moisture reduction. In this regard it is critical to note that many laboratories will present sample results on an "as is basis", implying that no correction for moisture content is made. For some tissue types (e.g. liver) the moisture content may vary significantly over different diseased states and the results should preferably be calculated on a dry matter basis.

In some cases reference values will make use of an animal specific moisture percentage correction for some tissue types. It is however highly recommended to present sample results on a dry matter basis as this allows for better clinical reference values to be obtained and compared over time. Although some laboratories will not routinely perform a moisture percentage or dry matter determination it is also possible to request the laboratory perform some moisture percentage determinations on a percentage of the samples submitted and to correct the "as is basis" results accordingly.

Samples may be obtained from appropriately acquired specimens with the correct authorisations and ethical clearances required in some instances. For commercial livestock samples may be obtained with the assistance of health inspectors or officials at abattoirs. Most commercial operations for animal production systems have post-mortem halls or facilities that also assist with sample acquisition.

With the notable exception of blood samples, in most instances samples may be excised, cooled and frozen for later analysis. In some cases the laboratory may request samples to be processed to some point, for example, drying and/or ground. Specific instructions should thus be obtained from the laboratory to be used.

Note:

- Ensure that the required protective clothing is worn to ensure biosecurity. Several zoonoses may be relevant and a specialist should be consulted prior to sample collection.
- Ensure that any necessary permits and/or permissions are acquired as needed.

4.10 A Case Study Approach of a Health Risk Assessment for Inorganic Chemicals as EDCs

4.10.1 Background

This section of the study aims to assess the feasibility of using a human health risk assessment approach to assess potential health outcomes, making use of the data described in the preceding section.

The focus of the parameters measured was to evaluate the inorganic chemical compounds with regard to their endocrine disrupting capability and any other adverse health effects anticipated.

For the purposes of this health risk assessment the four study sites were summarized as:

Site 1 = Surface Water: Komatipoort , Mpumalanga Province, chosen as it had water with low inorganic chemical content

Site 2 = Groundwater: Bonjanala, North West Province, chosen as an area representing water with high inorganic content

Site 3 = Municipal water: Springs, Gauteng Province, as a reference site making use of municipal water with low inorganic content (negative control).

Site 4= Kusile Power Station, Nkandala District, Mpumalanga Province, chosen as an area described as the largest construction area in South Africa, and which will continue to be developed in the future. Many known endocrine disruptors are expected to be found at this site (positive control).

Chemicals were found at the sites at concentrations predominantly below target guideline values, with the notable exception of the potential EDCs Bromide and Nitrate for Sites 1 and 2. As indicated in earlier sections these values are frequently observed at elevated values in agricultural water use sectors and mining effluents and as such is regarded as a priority inorganic EDC that varies significantly in groundwater across South Africa. Some anomalies were also noted in the water quality monitoring for Site 4, notably Fluoride, and other elements including selenium and arsenic (all EDCs) linked to coal deposits and coal mining activities.

Only outlier concentrations were considered for this part of the study. Herein we therefore used the most conservative restriction on the highest reported concentrations to establish a worst case scenario. This assists in prioritising the elements of interest and supports the bioassay screening process completed in the previous sections.
This section aims to assess the feasibility of using a human health risk assessment approach to assess potential health outcomes, making use of the data described in the preceding section 4 as the case study. The goal is therefore to determine whether endocrine disrupting contaminants contained within drinking water collected from the selected sites posed a human health risk.

The human health risk assessment was conducted according to the methodology described in the previous section. The framework consists of the following components;

- Hazard identification
- Exposure assessment
- Risk characterization

The risks were calculated using the following analytical tools:

a) Chemical analysis.
b) *In vitro* bioassay measuring oestrogenic activity

c) *In vivo* bioassay comparing the histopathology of broilers [1] from the sampling sites to a reference site.

T47D-KBluc bioassays were conducted to determine the oestrogenic activity in the collected water samples. The results of these tests were compared to the trigger value described in the previous section. Making use of the chemical analysis and quantitative health risk assessment, preliminary chemicals may be identified which pose threats to the reproductive endocrine system.

For the Broiler bioassay, the histopathology of the broilers from the various sites was compared to that of the reference site 3. The histopathology was examined by a specialist pathologist to determine if broilers demonstrated thyroid dysfunction. Although the results for the bioassay cannot be included into a quantitative human health risk assessment, they act as sentinel species, and provide an indication of possible thyroid disruption and potential adverse health effects.

The general approach was also detailed in section 3.4 of this report.

Table 40: The chemical compounds measured with chemical analysis, including those of suspected endocrine disrupting activity

	Analysed for	**Suspected endocrine disruptor**
Metals	Arsenic, chromium, molybdenum, vanadium, antimony, copper, strontium, beryllium, lanthanum, thallium, cobalt, nickel, zinc, iron, tellurium, bismuth, lead, tin, cadmium, manganese, tungsten, mercury, uranium, barium titanium selenium, caesium, boron	Arsenic , vanadium, selenium, manganese, iron, strontium, mercury, lead, cadmium, barium, antimony, chromium
Major cations	Sodium, potassium, magnesium calcium, lithium, ammonia	lithium
Major anions	Nitrite, sulphate, fluoride, Iodine, nitrate, chloride, bromide, bicarbonate, phosphate, carbonate	Nitrate, bromide, fluoride, iodide

4.10.2. Hazard Identification

During the monitoring procedures of the selected sites various inorganic chemicals and metal concentrations were determined. Of these values, the outliers were used to for the current risk assessment in order to describe a worst case scenario. The metals and inorganics identified as outliers at the various sites are briefly described. For all of the elements WHO guidelines are set, and reference doses (RfDs) have been either taken directly from the USEPA IRIS toxicology sheets, or from sub-species thereof. The risk parameters, formulae and symbols used for the risk characterization are given in Table 41.

[1] Broilers are chickens bred and raised specifically for meat production. The use of poultry as sentinels for public health is also used in the USA.

Table 41. Risk assessment formula and parameters.

Non Cancer toxic effects	**Carcinogenic toxic effects**
(1) ADD = C(m) × IR × ED × EF / (BW × AT)	(3) LADD = ADD × BW/ LT
(2) HQ = ADD / RfD	(4) Risk = LADD × β
Where **HQ** = Hazard Quotient **ADD** = Average Daily Dose ($mg^{-1}kg^{-1}day^{-1}$) **RfD** = Reference Dose **C(m)** = Contaminant concentration (mg) **IR** = Ingestion rate ($kg\ day^{-1}$) **BW** = Body Weight (kg) **ED** = Exposure duration (years) **EF** = Exposure Frequency ($days\ year^{-1}$) **AT** = Average Time (days)	Where LADD = Lifetime average daily dose (mg-1kg-1day-1) Cancer risk LT = Lifetime β = Slope factor as given by USEPA
Exposure parameters	
Events per year 350	Lifetime 70 years
BW 70 kg	
Ingestion rate Water ($1\ L\ day^{-1}$)	

The inorganic species likely to occur in the collected samples are briefly discussed.

Arsenic (As)

Arsenic is naturally occurring and widely distributed in the environment. Higher occurring concentrations of arsenic are primarily due to chemical waste disposal, smelting or mining of metals, fossil fuel combustion, and pesticide use. Arsenic is a metalloid and therefore does not break down in the environment, but can change from one chemical species to another. Arsenic compounds are generally soluble in water and do not evaporate. The toxicity of arsenic, like most metals, is closely linked to it speciation. Fish and shellfish accumulate the less toxic organic arsenic in their tissues, whereas humans can be exposed to significant inorganic arsenic concentrations in their working environments.

When ingested, high levels of inorganic arsenic can be fatal (60 mg/kg or 60 mg/L). Chronic arsenic exposure can cause tissue damage resulting in organ and possible vascular complications, Hyperpigmentation, keratosis as well blood changes. The oral reference dose for chronic exposure to arsenic in drinking water is 3 x 10-4 mg/kg-day. Confidence in the principal study on which the dose is based is as well as the RFD itself is considered medium.

Arsenic is classified as a Group A human carcinogen by U.S. EPA supported by case studies and epidemiological reports. The Ingestion of inorganic arsenic can increase the risk of cancer of the skin, lungs, bladder, and kidneys. The oral slope factor of 1.5 $(mg/kg\text{-}day)^{-1}$, is based on the increased incidence of skin cancer in humans who consumed drinking water with high arsenic concentrations. Although the study included a large number of people, uncertainties about the dosages of arsenic led

the U.S. EPA administrator that the slope factor may be modified downward by as much as an order of magnitude relative to estimates for most other carcinogens [86] [87].

Manganese (Mn)

Manganese is metal released into the environment from both natural and anthropogenic sources. Anthropogenic releases generally include, iron and steel manufacturers, power plants and coke oven emissions but also include runoff containing fungicides and dissolved fuel oils [88].

Oral and inhalation exposure may result in adverse neurological effects, of which manganism is the most well-known. Manganism is symptomized by tremors, difficulty walking and facial muscle spasms. No acute-, intermediate-, or chronic duration oral minimal risk levels have been derived for inorganic manganese. The EPA (IRIS 2011) derived an oral reference dose (RfD) value of 0.14 mg/kg/day manganese from all oral exposures. The confidence stated by the EPA in the studies on which the RfD is based as well as the RfD itself, to be medium. Studies were conducted in large populations over extended times, warranting an uncertainty factor of 1. Currently there is no evidence that manganese is carcinogenic, and the USEPA has classified manganese not to be carcinogenic to humans (Group D).

Bromine (Br)

Bromine is naturally occurring in water and living organisms and it reacts with other compounds to form bromides. Bromide commonly exists as salts with sodium, potassium and other cations, which are usually very soluble in water. Concentrations of bromide in fresh water typically range from trace amounts to about 0.5 mg/l. Increased anthropogenic inputs have, however, significantly increased the amount of total bromine humans are exposed to. Bromine is used in pesticides, cleaning agents, disinfectants and water [89]. Bromine is corrosive to human tissue in a liquid state and its vapours are very toxic with inhalation. The most important health effects that can be caused by bromine-containing organic contaminants are malfunctioning of the nervous system, damaging the thyroid gland and disturbances in genetic materials. But organic bromines can also cause damage to organs such as liver, kidneys, lungs and milt and they can cause stomach and gastrointestinal malfunctioning. Some forms of organic bromines, such as ethylene bromine, can even cause cancer.

Acute toxicity specific to bromine are not available. Bromide is, however, classified as Toxicity Category III for oral toxicity. The reference dose for the well-known organic bromide fungicide, methylbromide, is 0.0014 mg/kg/day based on epithelial hyperplasia of the forestomach in a rat study (USEPA, 1991). The associated uncertainty is a factor of 1000, but the confidence in the study and resulting RfD is medium. Methylbromide is however not classified as a human carcinogen.

Sodium bromide, also used as a pesticide and anti-bacterial cleaning agent, increases serum thyroxine and triiodothyronine in rates [90]. High levels of bromide in animals impact on the iodine metabolism in two pathways [91]: by a decrease in iodide accumulation in the thyroid and skin (and in

the mammary), and by a rise in iodide excretion by kidneys. By accelerating the renal excretion of iodide, excessive bromide can also influence the pool of exchangeable iodide in the thyroid. Interaction of bromide with iodide uptake by the thyroid gland most probably is the underlying mechanism leading to thyroid dysfunction and consequently to the observed alterations in the pituitary-thyroid axis. Sodium bromide is easily converted to sodium bromate in acid waters. The oral reference dose for bromate is 0.004 mg/kg/day based on renal defects in rates and presents with an uncertainty of 300.

Iron (Fe)

Iron is used to produce steel, magnetic alloys, pigments, polishing compounds, catalysts, feeds, disinfectants, and sewage and industrial wastewater treatment chemicals. Iron is also an essential nutrient; required for maintenance of good health. However, if ingested in larger quantities iron can be toxic, causing effects such as irritability, seizures, abdominal pain, vomiting, diarrhoea, lethargy, and coma. Humans do not have a mechanism to increase the excretion of absorbed iron in response to elevated body levels. Chronic ingestion of high levels of iron causes an increase in tissue iron levels. During iron overload, excess iron is stored in the liver and other organs. Massive iron overload can lead to liver cirrhosis and damage to other organs including the heart, endocrine glands, and pancreas.

A provisional oral RfD has been developed for iron based on typical dietary intake. The average intakes of iron, which range from 0.15 to 0.27 mg/kg-day do not cause iron overload, yet are sufficient to protect against iron deficiency. Dividing the NOAEL of 0.27 mg/kg-day by an UF of 1 yields a provisional chronic oral RfD of 0.3 mg/kg-day (US EPA, 1999). While confidence in the critical study is high, overall confidence in the overall database is medium because the data are insufficient to determine the chronic dose level that is associated with adverse effects in health individuals. This RfD may not be protective of people with disorders of iron metabolism and could be conservative if applied to forms of iron with low bioavailability. There is no evidence that iron can cause cancer.

Strontium (Sr)

Strontium has a variety of commercial and research uses. It occurs in four stable isotopes, of which the strontium-90 poses the largest concern. Strontium-90 has been used as an isotopic energy source in various governmental research applications, including in radiothermal generators to produce electricity for a variety of purposes including devices to power remote weather stations, navigational buoys, and satellites. Strontium-90 concentrates in bone surfaces and bone marrow, and its relatively long radioactive half-life (29 years) make it one of the more hazardous products of radioactive fallout. Bone tumours and tumours of the blood-cell forming organs are the main health concern. These tumours are associated with the beta particles emitted during the radioactive decay of strontium-90 and yttrium-90 [92].

In addition to potential radiogenic effects, strontium has been shown to inhibit calcification and cause bone deformities in animals, notably at high doses. Exposure to high levels of stable strontium can result in impaired bone growth in children. The EPA reference dose (RfD) for estimating the potential for non-cancer effects from oral exposure to strontium is 0.6 mg/kg-day. This value was based on rachitic bone observed in a rat study. The RfD and study were met with medium confidence and a uncertainty of 300. The effect of stable strontium on reproduction in animals is not known. No developmental studies in humans or animals examined the effect on the foetus when the mother takes in excess strontium.

The average annual concentration of 90Sr in water supplies should not exceed 8 pCi/L (0.3 Bq/L). EPA also established maximum contaminant levels (MCLs) in drinking water for radionuclide activities to protect against harmful effects of 90Sr. For beta particles like strontium, the MCL is 4 mrem per year (4x10-5 Sv per year). Although it is established that radioactive strontium may cause cancer as a result of damage to the genetic material (DNA) in cells, an oral slope factor for strontium has not been established.

Fluoride (F)

Fluoride salts occur naturally in the environment, the most common of which is sodium fluoride primarily used in dental care. Sodium fluoride and other fluoride compounds, such as fluorosilicic acid and sodium hexafluorisilicate, are used in the fluoridation of public water. The main health concern regarding fluoride is likely to be from excessive chronic oral exposure in drinking water. Significant amounts of fluoride deposit into bone, therefore, the primary target system for intermediate and chronic exposures of both humans and several laboratory animal species is the skeletal system (including teeth). Small amounts of fluoride in the diet can help prevent dental caries and strengthen bones, but there are a number of adverse effects that chronic ingestion at high doses can have on human health, including dental fluorosis, skeletal fluorosis, increased rates of bone fractures, decreased birth rates, increased rates of urolithiasis (kidney stones), impaired thyroid function, and lower intelligence in children [93].

Studies have shown that children with or even without dental fluorosis from exposure to excess fluoride, either through drinking water or through other sources, may have thyroid hormone derangements that may not be clinically overt until late stages [94]. In severe cases of crippling fluorosis, complete rigidity of the spine can occur, however, extreme cases are generally associated with malnutrition.

The reference dose for fluorine (soluble fluoride) in drinking water was initially set as 0.06 mg/kg/day in 1989 by the USEPA, and has recently been adjusted to 0.08 mg/kg/day (2011) based on severe dental fluorosis. The reference dose has been met with some controversy, as thyroid effects were reported for doses below 0.05 mg/kg/day, which were depended on sufficient iodine intake. The

reference dose should therefore be even lower to protect populations with iodine deficiency. The conservative reference dose used in the current case study was therefore 0.04 mg/kg/day.

Fluoride concentrations between 0.5 and 1.5 mg/L promotes development of strong bones and teeth, fluoride concentrations > 4 mg/L promotes dental and skeletal fluorosis and over 10 mg/L can cause crippling skeletal fluorosis and possibly cancer. No studies have addressed whether low levels of fluoride will cause birth defects in humans.

4.10.3. Quantitative Health Risk Assessment

To determine the possible risks posed by elements within the collected water samples, the maximum outlier concentrations distributed over the sampling areas were evaluated. Therefore the maximum concentrations found were used to describe a worst case scenario i.e. the levels posing the highest possible health risk. These outlier concentrations are summarized in Table 42.

Table 42. Concentrations of chemicals in water (mg/l) used in risk assessment.

	Metal concentration in water(mg/l)							
Site	**Br**	**As**	**Fe**	**Mn**	**NO3**	**Se**	**Sr**	**F**
Site 1	0.700	BD	1.100	0.100	BD	BD	0.300	BD
Site 2	3.300	0.070	0.200	BD	98.000	0.080	2.500	1.400
Site 3	0.043	BD	0.300	BD	2.000	BD	0.100	BD
Set B	0.140	0.074	2.800	0.450	2.000	0.078	BD	9.000

The average daily dose (ADD) determined with equation (1) in Table 1 for the consumption of 1 L of water by a person of an average body weight of 70 kg. The ADD for the identified outlier elements concentrations in Table 40 are given in Table 43.

Table 43. Average Daily Dose (ADD) (mg/kg/day) based on worst case scenario and consumption of 1 l of water.

	Average Daily Dose (mg/kg/d)							
Site	**Br**	**As**	**Fe**	**Mn**	**NO3**	**Se**	**Sr**	**F**
Site 1	0.010	0.000	0.016	0.001	0.000	0.000	0.004	0.000
Site 2	0.047	0.001	0.003	0.000	1.400	0.001	0.036	0.020
Site 3	0.001	0.000	0.004	0.000	0.029	0.000	0.001	0.000
Set B	0.002	0.001	0.040	0.006	0.029	0.001	0.000	0.129

The non-carcinogenic health risks posed by the outlier elements identified in each of the sites are shown in Figure 5. A Hazard Quotient (HQ) higher than 1 is acknowledged as an unacceptable risk by the USEPA, requiring further evaluation.

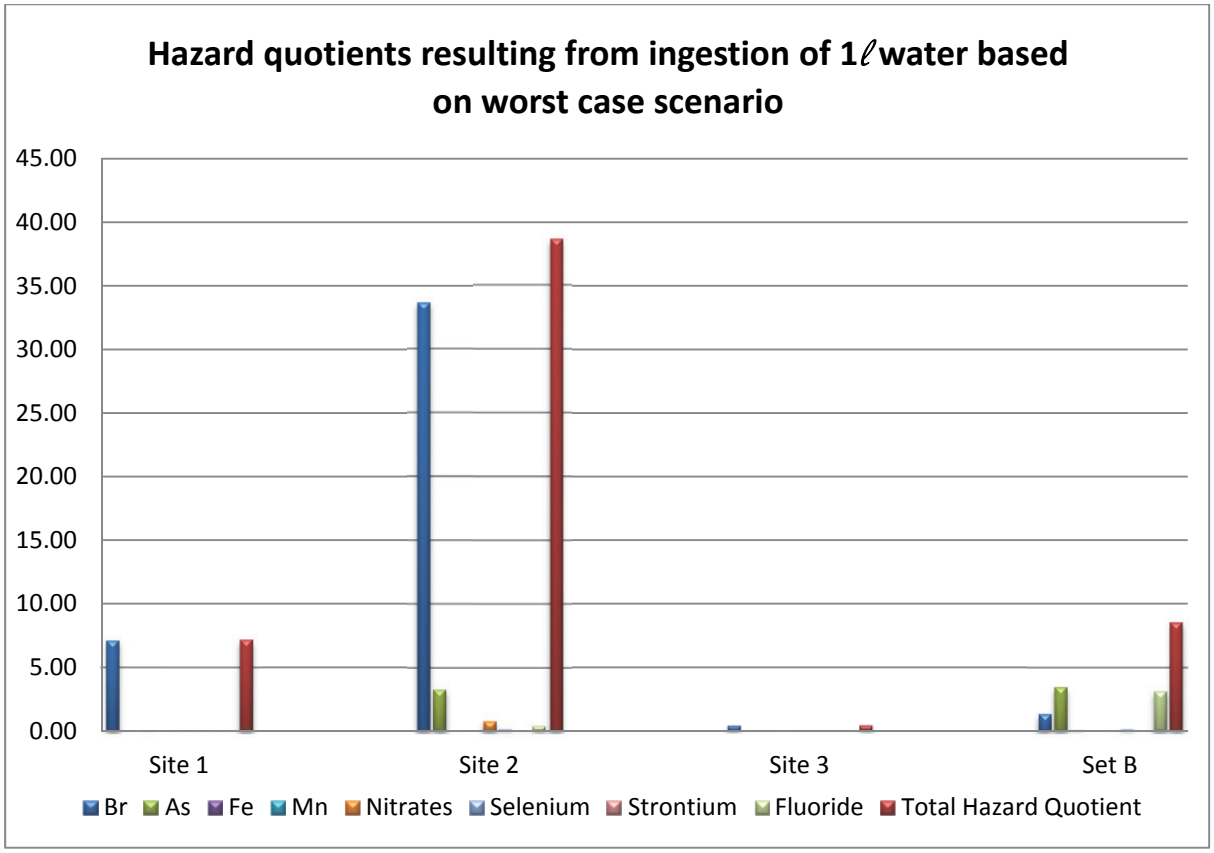


Figure 5: The hazard quotient for the elements identified in the worst case scenario for each of the sampling sites.

The elements detected in water samples collected from Site 3, pose very little health risk, and with regards to the evaluated species, is considered safe for consumption with no expectation of adverse health effects. The largest non-carcinogenic health risk posed at Site 1 and Site 2, is from exposure to bromide with a smaller risk posed by Arsenic at Site2. The total HQ for Set B, however, is a fair combination of Fluoride, Arsenic and a smaller contribution from Bromide exposure. Water collected from Site 2, poses by far the largest health risk.

Determining the LADD using equation (3) and the ADD calculated for arsenic concentrations in water, the carcinogenic risk (using slope factor of 1.5) posed by arsenic at site 2 and Set B was 6.4E-4 and 6.8E-4 respectively. These risks exceed the acceptable risk of 1E-06 set by the USEPA.

The largest threats are therefore posed by bromide and arsenic contamination. Critical evaluation of the calculated HQ is now required. This requires accurate review of the available toxicity studies of Bromide and Arsenic, consideration of the uncertainty associated with the determined RfD as well as

identification of possible endocrine effects. As many of the published studies did not specifically evaluate for endocrine activity, identified threats may pose a risk even at lower concentration. The following considerations are noted for the current case study:

A) The RfD used for the determination of bromine HQ was based on that of the organic compound, methylbromide. As with most chemical elements, the toxicity of bromine is strongly determined by its speciation. The RfD for inorganic bromide compounds (such as sodium bromide) is 0.7 mg/kg/day and for organic bromide compounds such as methyl bromide or ethyl bromide, is far lower and range from 0.009 to 0.0014 mg/kg/day. In order to conclusively determine the toxicity posed by bromide measured in the water samples, organic and inorganic speciation is required. As the impact of bromide on the thyroid is well established, endocrine activity associated with bromide contamination is more than likely at Site 1 and Site 2.

B) The USEPA (1998) slope factor for arsenic is 1.5 and is based on sufficient evidence that inhalation and ingestion of arsenic lead to increased occurrences of lung and skin cancer in humans. Arsenic interferes with glucocortoid signalling necessary to turning on genes involved in tumour suppression and other activities [95]. By preventing these genes from turning on, arsenic may increase the risks of cancer. Recent studies have supported As is a potent endocrine disruptor, altering gene regulation by the closely related glucocorticoid, mineralocorticoid, progesterone, and androgen steroid receptors (SRs) at concentrations as low as 0.01μM (~ 0.7 ppb). Recent studies have also shown that arsenic also disrupts the oestrogen receptor [96].

C) There remains substantial controversy on the acceptable levels of fluoride in drinking water. The NRC report (cites many endocrine effects of fluoride exposure, including decreased thyroid function, impaired glucose tolerance (Type II diabetes), and earlier sexual maturity. These effects are achievable at levels lower than the guideline value of 4 mg/L. In humans effects on thyroid function were associated with fluoride exposures of 0.05-0.13 mg/kg/day when iodine intake was adequate and 0.01-0.03 mg/kg/day when iodine intake was inadequate [97].

4.10.4 Endocrine Health Risk Assessment

Inorganic EDCs

Risk assessment using the reported element concentrations suggest that bromine, arsenic and fluoride may present possible health risks in the water samples collected from Site 2, and to a lesser extent at Site 1 and Set B.

These elements also have well documented endocrine effects, primarily targeting of thyroid functions. The determined HQ and review of toxicity data, suggest that these waters do pose a possible

endocrine risk, and that further evaluation may be required. It should be emphasized that hazard risk assessment describes an overall health risk and do not quantify the endocrine effects.

Although it is assumed that the reference dose is a conservative measure, many studies used to derive these values did not investigate endocrine activity. RfD do not consider life stages when protecting for endocrine effects which can be observed at much lower doses spread over longer periods, or at crucial development phases.

In vitro bioassay – oestrogenic activity

The T47D-KBluc bioassay responses in water samples for site 1, site 2 and site 3, were below the trigger value for oestrogenic activity (Table 33 RB5). Although these water samples indicated very little oestrogenic and androgenic activity (Table 29 RB1), other endocrine activities may be present in the collected water samples. The initial bioassay screening of the water samples therefore indicated low endocrine activity.

In vivo bioassay for thyroid activity – broiler histopathology

Broiler[2] sites were included at Sites 1, Site 2 and Site 3 (Reference site). The broilers were of the same breed, nutritional programme, health regime and environmental controls with the exception of atmospheric exposure and drinking water. The observed plasma thyroxine (T4) values for Sites 1 and 2 were significantly lower than those observed for the control Site 3. The lower T4 values suggest hypothyroidism and this was supported by the histopathological examination. It is likely that the thyroids of the poultry at Sites 1 and 2 experienced fundamental disruption, as evidenced by growth rate decreases in those production sites during the last week of the growth cycle.

Chemical analysis of the deep pectoral and liver tissue revealed that Site 2 presented with higher Br, Se, Pb, and I concentrations compared to Sites 1 and 3. This accorded with the water quality assessments in terms of anomalies and concentration ranges noted and possible mechanisms were described in the preceding results discussion. Since the production systems for Site 2 and 3 represent closed environmentally controlled houses and no air-based application of bromide-containing chemicals are applicable, the elevated Br concentration in tissues for Site 2 is most probably related to the high Br concentration in water. Since Site 1 represents open-sided houses in close proximity to sugar cane crops additional exposure to air-borne pesticides would need to be confirmed by air monitoring studies.

Whilst the chemical analysis of Sites *1* and 3 were not similar for key elements such as arsenic, selenium and iodine, it should be appreciated that the tissue results assist predominantly with ruling out major mineral disturbances linked to nutritional challenges, and are not solely intended for

[2] Broilers are chickens bred and raised specifically for meat production. The use of poultry as sentinels for public health is also used in the USA.

diagnostic purposes relating to either marginal disturbances or EDC mechanisms. There use as a baseline description is beneficial, similar to the perspective gained when investigating iodine deficiency disorders in community health.

Given the complexities of multiple exposure to the anomalies observed in the water quality assessments caution is required when using the tissue values observed to describe causation, but the results do provide some insights as to possible further research requirements. Additionally, it should be noted that the broilers from Site 1 are largely sold to rural villagers in the immediate vicinity, with the trace element values thus assisting with dietary exposure to several elements in terms of the maximum acceptable levels to consumers (e.g. arsenic and lead). Since they also provide the ability to observe trends over time they may assist with evaluating the efficacy of mitigating and risk management strategies.

Although it is clear that the broilers at Sites 1 and 2 were exposed to thyroid disrupting chemicals, it should be noted that the objectives of the case study were not to definitively determine the responsible compounds/groups of compounds nor to establish the relative contributions from different exposure routes (e.g. drinking water or inhalation). The exposure from drinking water could be established as water intake is recorded on a daily basis with body weight on a weekly basis. The conclusion is however made that combined with the quantitative health risk assessment and *in vitro* bioassays, it is evident that the community living in Site 2 are being exposed to EDCs. The production parameter problem descriptions (growth-related), histopathology and clinical biochemistry results for Site 1 would also suggest a similar conclusion could be reached.

As detailed in the preceding sections the focus of the Manual is on water quality in terms of EDCs. A key objective was to use the methodology employed to assess if potential EDC exposure in the form of water quality could include an additional step that could provide guidance on whether further investigations for community health, or other relevant receptor types in a catchment, were indicated.

Compared to only conducting bioassays in water and sediment the broiler sentinel approach does reduce exposure variation and assist with investigating the combined outcome of all exposures in the form of clinical and histopathological evidence. The sentinel broilers present a method with which to determine if a community is facing risks posed by EDCs.

These studies can present specific information of the exposure route from drinking water, but since air quality and exposure factors are at present not performed on a routine basis for EDC aspects, the studies cannot result in any health risk quantification. The subject of air quality monitoring is being investigated for suspected EDCs in a separate WRC Project (K5.1956). When thyroxine studies are supported by tissue analysis, accumulating compounds may be identified, which can be compared to values determined in drinking water. Again, these methodologies are intended to be indicative and to assist in directing further human health risk investigations.

Thus, although it may seem a simple task to thus translate endocrine disruption to a physiological significance the numerous management factors relevant often render differential diagnosis thereof complex. The combination of EDC exposure media data, blood hormone values and histopathology are thus recommended to contribute towards confidence in both diagnostics and the decision to accord priority for further investigation to specific sites.

4.10.5 Recommendations

A number of issues are needed to better understand whether endocrine disruptors are present in water samples. It is therefore recommended that the following steps be considered;

- Further monitoring of the water at the selected sampling sites.
- Speciation of the chemical compounds found to be of concern.
- Organic chemical analysis in order to determine the presence of larger organic compounds such as pesticides and pharmaceuticals.
- Constant review of updated toxicological studies for observed endocrine effects caused by the elements identified in the water samples.
- Communicating of potential risk to communities if warranted by sufficient evidence. The primary objective of risk communication is to promote awareness and understanding of specific health issues as well as the risk management strategy. The methodology of the communication of risk to affected communities is described in Genthe and Knoetze (2008) [40]. Herein, the issues, principles and barriers to effective risk communication are defined. An appropriate strategy can be devised and guidance is given on presenting prepared information. The roles of the media and how to effectively use them in a risk communication strategy is also discussed.
- Gaining community involvement. Given sufficient evidence of an ED threat and an effective communication strategy is in place, support and participation from the local community may be included in long term monitoring and risk assessments. Examples of community involvement include;
    - Voluntary health surveys. Herein, risk assessors have questionnaires on self-diagnosed health issues such as diarrhoea, memory loss, tiredness, menstrual cycles, weight issues etc.
    - Voluntary water-use surveys. How do communities use their water? Are they in some ways increasing their exposure?
    - Communities may also be able to identify possible effluent in-flows into the natural resources unknown to the risk assessors.

5 CONCLUSIONS

5.1 Main Conclusions

The case studies conducted highlighted several key issues relevant to EDC investigations. It should be considered that the objective was to investigate the ability to use the various aspects of the volumes of the EDC Manual to meaningfully conduct EDC research and that a primary goal remains to provide some statement on the hazards and risks posed by water quality regarding EDC exposure.

Currently a major challenge that remains is that any upper limit set would largely be based on bioassay screening tests and possibly be supported by analytical confirmation of a potential causative compound. Final target limits for management purposes would thus remain subject to criticism regarding the lack of clear evidence of actual exposure in the affected receptor (e.g. identified community) and any clear evidence of an adverse endocrine event.

Stated differently, if a community drinking water supply yields an observation of a bioassay response of activation or inhibition and the analytical detection of an EDC in the water, it still does not imply that endocrine disruption will occur following exposure. Even if some public health study is conducted the major constraints in this regard remain time delays in obtaining ethical clearance, the high cost of such studies and the reality that evidence of endocrine disruption does not in itself imply physiological significance or subsequent adverse effect. A common criticism of most public health studies remains true for EDC issues, namely difficulties in not only describing, but also in correcting for, confounding factors.

These are significant hurdles that are apparent in research conducted in other public health issues and particularly relevant for EDC studies due to the complexities of the physiological mechanisms involved.

It may also be argued that the objective should not be to set a limit based only on a precautionary principle as this may result in limits that will simply never be met. This approach is fundamental to water quality guidelines and well described in the current WHO drinking water quality standards (http://whqlibdoc.who.int/publications/2011/9789241548151_eng.pdf). In the derivation sections for some water quality constituents the consideration of both laboratory detection limits and reasonably achievable and available treatment technologies form an integral part of setting a guideline limit.

It is also prudent to differentiate between those EDCs that can be managed in terms of pollution reduction and those that are inherent. Considering the reliance on subterranean water in South Africa it may also be argued that the description of those naturally occurring EDCs should be a priority as these exposures will in all likelihood remain for a significant period of time. The objective of EDC research should thus be able to be sufficiently descriptive as to allow for some risk factor identification that will allow for meaningful management actions to be formulated.

5.2 Key Recommendations

The methodology employed for these case studies have provided some key information that may assist towards answering these data gaps and methodology challenges. These may be summarised as:

- **Procedural – Use of Sentinels**
  - The use of broilers as a method for investigating the potential outcome from exposure to EDCs yielded the following key observations:
    - The benefit of being able to describe conditions regarding source (dietary, water, air quality, health programme, production conditions, common genetic production parameter standards) assists with confidence in interpreting observations between exposure and effect.
    - Not all exposure pathways or sources were analysed for these case studies which prevented a full quantitative risk assessment from being performed, but this could be done for water quality as intake (water quantity ingested), concentrations (water quality) and water turnover rate (body weight correction) data are available at the majority of production sites. Air quality and exposure determination remains challenging for EDCs but is currently being investigated in a separate WRC Project (K5.1956).
    - The low variation between production inputs assists with comparisons between observation sites.
    - Where differences do exist they were potentially related to water quality. The supporting evidence gathered allows for priority to be assigned between the investigation sites and potential EDC exposures.
    - It remains a challenge to obtain recent reference documentation for many EDCs, as many may not have been recently updated for the specific chemicals (e.g. US EPA IRIS Toxicology Sheets) yet have recent scientific publications in peer-reviewed journals indicating their role as potential EDCs.
  - The observation of endocrine disruption was supported by meaningful clinical data, including clinical production issues, blood hormone values, endocrine gland histopathology and multiple tissue composition analyses. These were found to be:
    - Cost effective.
    - Easy to perform and thus repeatable.
    - Attractive from a research perspective as similar genetic breeds and production system dynamics are available throughout South Africa.
    - Provide an additional benefit as in many cases the broilers are themselves consumed by members of affected communities and their description (composition) assists with exposure assessment for public health.

Procedural – Monitoring and Assessment

- The use of the Eskom/Kusile Power Station highlighted the following issues:
    - It cannot be assumed that EDC constituents will automatically be considered in Water Use Licence and Environmental Authorisations, despite their presence as pollutants implying this.
    - As a general summary statement on the lessons learnt it may be noted that:
        - Environmental Consultants are largely unaware of the EDC issues relevant to the various receptors that may be impacted upon.
        - Application of the current DWAF (2006) and SANS 241:2011 water quality guidelines and standards are poor with the context of target water quality range and discharge compliance mostly not understood.
        - Inclusion of EDCs linked to potential sources in terms of EIAs and WULAs should be highlighted for both:
            - Regulatory Authorities
            - Scientist and consultants working in environmental and related fields
- Human Health Risk Assessment:
    - Although the methodology employed is generally adopted internationally, it is reliant on practices that originate from countries (e.g. US and Europe) that have different environments. Some of the default values and approaches may not be entirely appropriate for South African conditions, where factors such as geology and groundwater chemistry, actual water intake values in certain community and workplace sectors, may alter the final outcome of both hazard and risk assessments. When this is viewed within the context of the uncertainties concerning EDC cause and effect relationships, developing assessment methodology that caters for the site-specific risk factors relevant to the South African environment to a greater extent would appear to be a valid approach.

- **Case study Contributions**
    - Following the inputs from this project and deliverable to the EMC process at the Eskom/Kusile Power Station it now conducts one of the most comprehensive EDC monitoring and assessment programmes for a catchment in South Africa. New techniques developed for a separate WRC Project (K8/999) have been included as standard methodology.

    - Eskom/KPS have offered access to interested researchers to the monthly monitoring reports of groundwater, surface water and air quality, and to ongoing aquatic studies in the affected wetlands.

    - The methodology developed for the broiler sentinel sites is viewed as having assisted

with investigating a potential EDC effect due to naturally occurring geochemical anomalies in groundwater, but also having highlighted the physiological mechanism and significance of the EDC effect.

5.3 Research Needs

Research needs applicable to the different volumes of the EDC Manual are all relevant in terms of identifying further work to be conducted. As has been the case in the methodology adopted for this volume, these needs are multidisciplinary and inter-related.

With specific reference to this volume, the following research needs are highlighted as:

- The use of the Eskom/KPS monitoring data by other EDC research projects is encouraged. This includes the immediate environment and affected communities and other related studies in the broader catchment. Increased collaboration and involvement of researchers with experience in EDC research is a key requirement to this site being able to add beneficial knowledge to EDC matters. As the largest construction site in South Africa with sensitive and priority wetlands potentially affected, this site represents a viable testing environment for EDC research for both monitoring and assessment and catchment management strategies.

- The use of the broiler production sites and similar sentinel approaches should be considered on a broader scale. Comparisons between commercial and rural community poultry projects would be of value. Although not an objective or within the scope of this deliverable follow-up studies are strongly recommended with regards to public health in the potentially affected communities. The use of this approach is currently underway in a separate WRC project on agricultural chemicals that also employs the bioassays developed for the EDC Manual (WRC K5/1956). Although air quality and inhalation exposure continues to be a required information type, viable methods for obtaining the required information remain under development. This aspect warrants attention in order to place perspective of risk from EDC exposure from multiple sources.

- Awareness needs to be increased within the regulatory authorities for the Department of Water Affairs and the Department of Environmental Affairs regarding EDCs. A similar need has been identified within various related specialist consultancy fields where EIAs and WULA are performed. This is a priority area and involvement from the various researches in the EDC field in terms of commenting and compiling proposed amendments to relevant Schedules to both the NWA and NEM:WA is strongly recommended.

As a final concluding remark it may be observed that the EDC Manual does appear to have a real need in terms of current knowledge transfer and methodology guidance.

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