WRC PROGRAMME ON ENDOCRINE DISRUPTING COMPOUNDS (EDCS)

Volume 4: Implementation of the Extended Research Plan on Endocrine Disruptor Chemicals in Water Resources

Report to the
Water Research Commission

by

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WRC Report No KV 234/09
ISBN 978-1-77005-949-8

March 2010

Obtainable from:

Water Research Commission
Private Bag X03
Gezina, 0031

The publication of this report emanates from a project entitled *Implementation of the Extended Research Plan on Endocrine Disruptor Chemicals in Water Resources* (WRC project K8/760).

This report forms part of a series of reports. The other reports in this series are:

Volume 1
WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 1. Strategic Research Plan for Endocrine Disrupters in South African Water Systems **(WRC Report KV 143/05).**

Volume 2
WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 2. Implementation of a Research Programme for Investigating Endocrine Disrupting Contaminants in South African Water Systems **(WRC Report 1402/1/08)**.

Volume 3
WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 3. Extended Plan for the Endocrine Disruptor Research Programme of the WRC, 2006 – 2010 (**WRC Report KV 228/09**)

FOREWORD

A number of substances, including some pesticides, have the potential to interfere with normal functions of the body, particularly the endocrine system that regulates physiological functions through hormonal signals. These substances, known collectively as Endocrine Disrupting Compounds (EDCs) include many natural and synthetic chemicals. While not all persist in the soil and water environment, many are classed as persistent organic pollutants (POPs). Most of the implicated chemicals are widely distributed in the environment and are found across national boundaries. Because of the accumulation of EDCs in water, the Water Research Commission (WRC) launched the EDC Research Programme to coordinate local research undertaken on this subject and also to involve other role-players such as government departments, industry and water suppliers in the research. Four projects, aimed at providing strategic guidance to the Research Programme, were undertaken:

1. WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 1: Strategic Research Plan for Endocrine Disrupters in South African Water Systems (WRC Report KV 143/05) of this report series, a strategic research plan was developed to guide research into the occurrence and levels of EDCs in South African water systems. This included the selection of chemicals to be monitored, the selection of methods to analyse for EDC activity as well as for individual compounds, determination of the capacity and capability of local laboratories to conduct analyses and the selection of a few sites where a surveillance study could be undertaken. A training programme was proposed as well as the framework for a limited surveillance study.

2. WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 2: Implementation of a Research Programme for Investigating Endocrine Disrupting Contaminants in South African Water Systems (WRC Report 1402/1/08), the implementation of this plan was described and the results obtained in the study were presented. The results proved that EDCs are indeed present in the four sites studied. It was indicated that EDC activity may have been caused by pesticides, industrial chemicals and/or natural and synthetic hormones. The report also confirmed that the analytical capacity to determine EDCs did exist in South Africa, although the analyses of a large number of samples may create a problem. Capacity building and the expansion of analytical facilities are priorities, not only to improve the country's international research credibility but also to provide job opportunities to post-graduate students. It is important that suitable tools should be developed to assist governing bodies in managing the problem of environmental pollution by EDCs. Very little information on certain aspects of EDC contamination is currently available and it is therefore necessary to conduct research in areas where knowledge is lacking.

3. WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 3: Extended Research Plan for the Endocrine Disruptor Research Programme of the WRC, 2006-2010 (WRC Report KV 228/09), an extended research plan was suggested to fill the knowledge gaps and address the needs of all stakeholders. The aim was to provide guidelines for future research to ensure that the needs of all the role players in the field of EDC research are addressed. It became clear that a policy needs to be developed to ensure proper management of the EDC pollution problem. A needs and gaps analysis was conducted at two workshops attended by all role players. At these workshops knowledge gaps were identified and several project themes for future research, including a time scale and budget, were developed in conjunction with the

researchers in the field. These projects cover a wide range of research subjects: the study of the physiology of selected sentinel species, the study of the effects of EDCs on the thyroid function and immune system and analytical methods to measure such effects, the occurrence of veterinary drugs in water systems, the effect that DDT may have on humans living in a DDT-spraying area and the development of a human health risk assessment model for EDCs. It was also proposed that future research should be undertaken on two levels: **technical research** (such as the projects described above), and **applied research** (research at ground level). An action plan was proposed for applied research to establish the impact on the environment of agricultural and industrial chemicals with EDC properties, as well as of chemicals resulting from human activity, such as natural and synthetic hormones, which are also polluting the environment.

4. WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 4: Implementation of the Extended Research Plan on Endocrine Disruptor Chemicals in Water Resources (this report), is the last in the series and reports on the implementation of the extended research plan as recommended in Volume 3. It gives an overview of the status of projects from 2006-2010 and recommendations for future research and actions to be taken in the WRC EDC Research Programme.

ACKNOWLEDGEMENTS

In order to determine the needs of stakeholders and to identify knowledge and research gaps, a workshop was held in Morgenson, Stellenbosch during 2003 which was attended by delegates from government departments, water suppliers, the environmental sector, industry and human health professionals.

A follow-up workshop held at Pumulani Pretoria in 2005 to discuss the initial EDC research programme and its outcomes and also to identify further actions.

The workshops were attended by the following:

Mrs APM Moolman	Water Research Commission (Chairperson)
Mrs A Burger	Private consultant (Programme manager)
Mrs A Albertus	Rand Water
Mr R Albertus	SASOL
Dr I Barnhoorn	University of Pretoria
Prof MS Bornman	University of Pretoria
Prof H Bouwman	University of North-West
Dr E Cairncross	Peninsula Technikon
Prof C de Jager	University of Pretoria
Prof HH du Preez	Rand Water
Prof OS Fatoki	University of Venda
Mr H Garbers	SABS
Mrs B Genthe	CSIR
Dr S Jooste	DWAF
Dr PL Kempster	DWAF
Mrs P Marais	ARC (PPRI)
Mr L Meintjies	TAU-SA
Dr J Meyer	University of Pretoria
Dr WL Muller	Rhodes University IWR
Dr T Naude	University of Pretoria
Mr SA Pieterse	Cape Metro Council
Dr E Pool	University of the Western Cape
Prof AJ Reinecke	Stellenbosch University
Mr V Schillac	AMPATH
Mr C Schoeman	Rand Water
Mrs B Sereda	ARC (PPRI)
Mrs JL Slabbert	CSIR
Mrs M Steyn	CSIR
Mrs A Swemmer	ARC Onderstepoort
Dr P Terblanche	MRC
Prof JHJ van Vuuren	University of Johannesburg
Prof JH van Wyk	Stellenbosch University
Mrs A Venter	CSIR

EXECUTIVE SUMMARY

A strategic research plan for Endocrine Disrupting Compounds (EDCs) in water resources of South Africa was proposed in 2001 and led to the development of the report, WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 1: Strategic Research Plan for Endocrine Disrupters in South African Water Systems (WRC Report KV 143/05). This plan was implemented during 2003-2005 and described in the report: WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 2: Implementation of a Research Programme for Investigating Endocrine Disrupting Contaminants in South African Water Systems (WRC Report 1402/1/08). The occurrence of EDCs in several selected water resources was confirmed and it was established that the contamination was caused by agricultural practice, mining and industrial activity as well as human excreta. During the execution of the project it became clear that both the aims of the EDC programme and the content need to be revised and extended to satisfy stakeholder needs and fit into the national plan for strategic scientific research. An extended strategic research programme was proposed in 2006: WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 3: Extended Research Plan for the Endocrine Disruptor Research Programme of the WRC, 2006-2010 (WRC Report KV 228/09). The aims of the plan were to:

- Provide government with a sound scientific base to develop a policy to address EDC pollution in South African waters
- Develop tools for scientists and other stakeholders to measure and determine the impact of EDC pollution from agricultural, industrial and human activity on the water resources of the country.

The aim of this project (Volume 4) was to determine to what extend the proposed projects have met the aims and objectives of the EDC extended research plan of the WRC as proposed in Volume 3 and to assist in planning future research where knowledge gaps still exist with the aim of meeting the stakeholder needs.

To develop a policy for the management of EDCs in South African water resources, government has to be able to make rational and informed decisions. Critical information, such as the magnitude of the pollution and the risk it poses to humans and animals, is essential. To provide the government with a sound scientific base to develop such a policy is no easy task. Several problems have to be addressed, e.g. the diversity of chemicals with EDC properties as well as the fact that very little is known about the mode of action of EDCs in the human body and in the environment. Furthermore, the development of a risk assessment model to forecast the effect that EDCs may have on human health and the environment is of vital importance.

In order to determine the magnitude and risk of pollution, the levels of the contaminants in the environment must be known. Unfortunately, only limited data exist on the actual levels of occurrence of EDC compounds in water resources. Since EDCs are active at very low concentrations and chemical analyses to detect such trace levels are very expensive, alternative methods for determining low levels of contamination by measuring the EDC activity, have to be developed. Methods for both chemical analysis and activity testing are already in place in several

laboratories at universities and other scientific organizations in South Africa. Furthermore, methods were developed and implemented to determine the mode of action of EDCs on the reproductive system of the human body as well as on the thyroid function. Several projects have also been proposed (and some have already started) to study the effects of EDCs on other hormone systems such as the nervous system and the immune system.

During workshops held at Morgenson, Stellenbosch and Pumulani, Pretoria, which was attended by stakeholders to identify existing knowledge gaps, there was consensus that the following were needed:

- Reliable and credible data on occurrence and magnitude of EDCs in South African water resources
- Information regarding the fate and behaviour of EDCs in the environment and in the human body
- Models for risk assessment on EDC contamination in water
- Relevant, affordable and rapid analytical methods to determine EDCs, and
- Facilities for analytical procedures.

To meet these demands it was recommended that future research should be divided into two levels:

- **Fundamental research**: Research projects aimed at understanding the mode of action of EDCs, new trends in analysis and development of risk assessment models and a model to predict the environmental fate of toxic organic chemicals in the aquatic environment**.**
- **Applied research**: Field studies to determine the impact of EDCs on water resources and the environment.

This report focuses on project themes which were developed and proposed in order to meet the needs of the stakeholders. It provides short summaries of progress reports on existing projects and lists a number of projects which have been proposed for future research.

Providing a sound scientific base to assist government in policy making (first aim)

It was concluded that good progress has been made in 2006-2008 to meet the extended aims of the programme. The **presence of EDCs** in water, sediment and biological tissue of aquatic and terrestrial animals was confirmed. It was furthermore established that agricultural practice, industrial and mining activity, human excreta and other natural occurrences were the main **sources of contamination**. **Detrimental hormonal effects of EDCs were observed** in aquatic and terrestrial animals as well as in humans. Studies concentrated mainly on the reproductive system, but some researchers found indications that the thyroid and immune systems were also affected. Humans and animals are not only exposed to EDCs through the ingestion of water, but also by exposure to food and inhalation of air. **The actual magnitude of EDC** contamination is not yet known and the impact on the environment can therefore not yet be established. **Health risk assessment** (in EDC research the term Effect Evaluation is more appropriate) depends on information regarding occurrence, magnitude of contamination, cause-effect data and other parameters such as exposure pathways and lifetime exposure. Although not enough information is currently available, this should, however, not prevent the Programme from moving into the

final stages of the extended research plan (Management and Remedial Action). Some of the research continued in 2009 and follow-up projects have been proposed after further consultation with stakeholders.

Providing tools for researchers to measure occurrence and magnitude of EDC contamination in the environment (second aim)

Data acquisition, fate and behaviour and risk assessment
During the "needs and gaps" analysis, it became clear that the availability of accurate, reliable and repeatable analytical results was top priority for all stakeholders especially in the field of activity testing (biological/biochemical analysis). It is of utmost importance that methods used for activity testing be standardized and harmonized with those used in other countries. The contact and cooperation with the Global Water Research Coalition (GWRC) should therefore continue and laboratories active in this field should be encouraged to participate in inter-laboratory trials.

A clear need for a set of guidelines on the planning and execution of EDC studies as well as on the management of EDC pollution, led to the decision to develop such guidelines aimed at researchers and tertiary institutions studying EDCs, and their behaviour in the environment, as well as for industries and catchment management agencies that need guidance on such studies or for management of pollution. This manual will include volumes on monitoring and assessment (Volume 1), a sampling guide (Volume 2), focus on a toolkit and analytical methods (Volume 3) and ultimately, provide management options (Volume 4) where EDC pollution has been detected.

Method development and calculation

As always, calculation of results obtained from biological/biochemical tests need to be handled with care since different ways of calculation and interpretation may lead to controversial conclusions. The issue of calculation is still worldwide a matter of contention. Workshops held in the past in South Africa on this subject did not produce positive results. It is therefore essential that a workshop should be held to clear up this issue, even as an addendum at a later stage when new initiatives could clear the uncertainties. The recommended methodology and calculation process should be presented in Volume 3, the analytical guide of the series, mentioned above. EDC studies require chemical analysis at very low levels in different matrices, which requires very sensitive methods and sophisticated instrumentation such as GC-MS and LC-MS. The development of rapid and cost-effective analytical procedures, both activity testing procedures and target analysis, should therefore continue. The water matrix is complex and EDCs from various chemical sources (pesticides, fabric softeners, flame retardants, plasticizers, anti-oxidants, personal care products, pharmaceuticals and natural and synthetic hormones) may be present in the resource at the same time. Because of the number of variables, it is virtually impossible to determine the impact of a certain group of pesticides in a water resource during a field study. Activity testing gives an indication of total EDC pollution, but gives no indication of which EDC or group of EDCs is present. A small number of EDCs may be determined by chemical analysis using multi-residue methods, but currently there are more than 400 chemicals suspected of endocrine disruptive (ED) activity and the cost for analyses could easily become

astronomical. Laboratory studies are therefore recommended to determine the potential impact of a group of chemicals suspected to be present in the water.

Risk assessment and remedial action

The WRC funded studies on the removal of EDCs and the estrogenic activity in selected drinking Water Treatment Processes, (WRC Report K5/1532/1/08) and Sewage Effluent (WRC Reports 1555 in 1402/1/08 and 1590/1/08) focused on the removal of EDCs. These studies have not tried to identify all the possible EDCs that could be in the source or treated water, but rather try to get some indication on the presence or absence of EDCs. Where water is circulated continuously, there may be a build-up of hormones in drinking water, but currently no other data exist on the levels of hormones in treated drinking water. These hormones, as well as pesticides and industrial chemicals with EDC properties, have been detected in rivers and dams across the country. Although the impact of these chemicals could not yet be established, detrimental effects have been observed on the hormonal systems of aquatic and terrestrial animals. Waste water treatment plants that are not conforming to effluent discharge standards could contribute to the load of EDCs and specifically hormones in the water resources.

However, humans are not only exposed to treated drinking water, but also to untreated water such as surface and ground water, the environment and food products containing EDCs. It is therefore important to include all water systems in a study. The aquatic animals are on the other hand continuously exposed to these loads.

Analytical facilities

Researchers are continuously frustrated with the lack of analytical facilities in the country. For international recognition of results, analyses in accredited laboratories are required. Only a few such facilities are currently available in South Africa. EDCs are active at extremely low levels and therefore require specialized and very sensitive methods to determine them at the relevant concentrations. Operating an ultra-trace laboratory not only requires very sophisticated instrumentation, but also highly qualified operators with years of experience in this field. EDC field studies also require the analysis of a large number of samples to make the results statistically valid. Only a few selected laboratories have the capacity to run the required number of samples if the specific laboratory is not equipped for this purpose. An effort should be made to establish and/or build more such facilities in the country. Forty-two students were trained at different educational institutions during the execution of different projects in the EDC research programme. Only two of them remained. Their main reason for leaving was poor remuneration and a shortage of permanent jobs. A strategy to establish accredited facilities in the country which will provide job and career opportunities to skilled staff is formulated and presented in this report.

Knowledge management

A number of research projects on EDCs were undertaken during the last ten years at various institutions and universities. Following a call by the WRC for a coordinated approach, some collaboration between researchers and post-graduate students involved in various research projects was established. A number of projects in the field of EDC research were also undertaken outside the WRC Programme by universities and organisations such as the CSIR and ARC. It is recommended that all relevant information, containing summaries and conclusions of all these projects, should be published and grouped together on the WRC website to ensure easy access to scientists working on this field.

Future of the WRC's EDC Research Programme

It is recommended that the WRC's programme on EDC research should continue, but in future the focus should be on the ***management*** of water resources, i.e. risk assessment and remedial action. Attention should still be given to the development of new analytical techniques and laboratory facilities for the detection of EDCs as well as on the removal of EDCs in sewage and industrial wastewater treatment plants. Special attention should also be given to capacity building and manpower development in this highly specialised field of research.

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1. INTRODUCTION

A strategic research programme for Endocrine Disrupting Compounds (EDCs) in water resources, Volume 1, of South Africa was proposed in 2001 (WRC Report KV 143/05) with the aim to determine the occurrence of EDCs in South African water systems in order to determine the associated human, animal and environmental health risk in South Africa and to give guidance to researchers and managers on research and identify knowledge gaps to get a better understanding of the EDC problem. This plan, Volume 2, was implemented during 2003-2005 (WRC Report 1402/1/08). The main aim of this phase and consortium of projects was to establish whether EDCs occur in South African water systems and should they be present, to determine the probable source of the contamination, and whether there is evidence of ED (endocrine disruptive) effects on humans and wildlife. The presence of EDCs can be detected by establishing their effect on biota using biological/biochemical means or by target analysis (chemical analysis). The majority of the research done was aimed at the effect of EDCs on the reproductive system and very little attention was given to the effect on other hormone systems such as the nervous system, the immune system and the thyroid function. It also became clear that the country did not have the relevant analytical methods and capacity to conduct a comprehensive study of EDCs in our water resources. This phase of the programme was therefore dedicated to the development of suitable analytical methods to determine EDC activity in water and to conduct target chemical analysis in water, sediment and biota. Inter-laboratory tests were conducted to ensure that the required low detection levels were reached and that results obtained by different laboratories were in agreement.

A small field study was conducted at four selected sites to determine the presence of EDCs in our water resources. The occurrence of EDCs in several selected water resources was confirmed and it was established that the contamination was caused by agricultural practice, mining and industrial activity, the lack of sanitation in certain areas as well as resulting from the fact that sewage works did not comply to recommended effluent standards. During the execution of the project it became clear that both the aims of the EDC programme and the content need to be revised and extended to satisfy stakeholder needs and to fit into the national plan for strategic scientific research. The main aim of the programme now changed to the *management* of the EDC pollution problem. The responsibility for water pollution management ultimately rests with the government, but the Water Research Commission (WRC) can play a critical role in providing the government with the necessary information and motivation so that a policy may be developed for the management of EDC pollution.

To manage the problem we need to know the **magnitude** of the pollution as well as the **risk** it poses to humans and the environment. Risk assessment in the environment requires an understanding of both the fate and behaviour of the specific compound in the environment and the associated effects on the biota. Activity testing (both *in vivo* and *in vitro*), as well as targeted chemical analysis, is therefore essential in obtaining sufficient information to determine the risk to humans and wildlife.

An extended and revised research plan, (Volume 3), was initiated in 2006 (WRC Report KV 228/09). The aim was to evaluate and review the preceding strategic plan (Volume 1) as well as the implementation of recommendations from Volume 2, to ensure that the EDC Research

Programme addresses all the relevant factors and that a more comprehensive picture of the endocrine disrupting (ED) effects on humans and wildlife could be obtained. A new extended strategic research plan was developed to include the needs of the stakeholders as well. In order to meet the aim of this extended strategic research plan, the following objectives were set:

- To provide government with a sound scientific base to develop a policy to address EDC pollution in South African waters
- To develop tools for scientists and other stakeholders to measure and determine the impact of EDC pollution from agricultural, industrial and human activity on the water resources of the country.

1.1 AIMS AND OBJECTIVES

The aim of this project was to implement the aims and objectives of the extended and revised research plan as defined in Volume 3. A schematic presentation of this plan is given in Fig 1. This framework was also discussed in detail in the Scoping Study to determine the Potential Impact of Agricultural Pesticides with ED properties on the Water Resources in South Africa (WRC Report 1774/1/08).

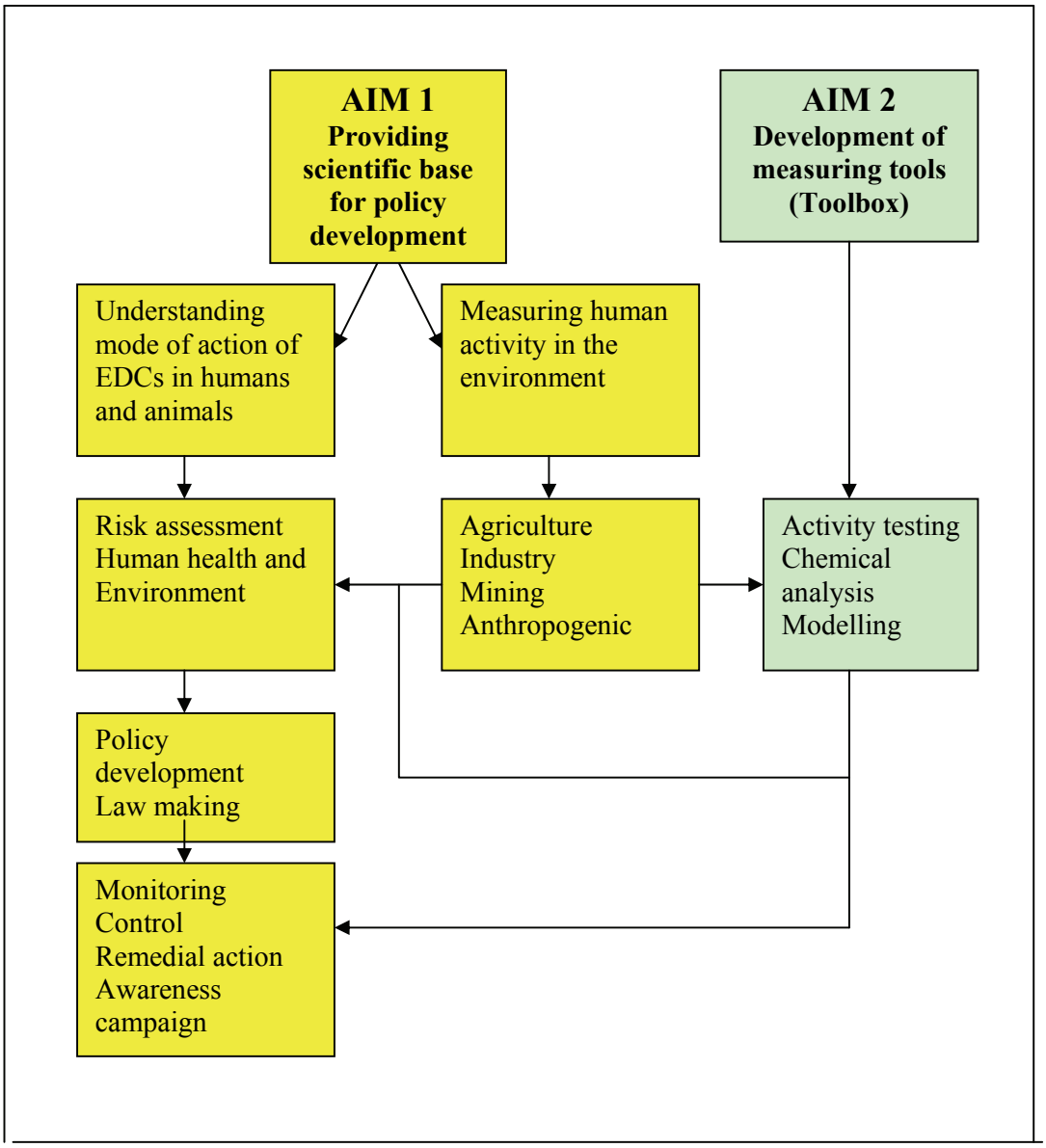

Figure 1: Framework for Strategic Research Plan for Endocrine Disruptor Chemicals in Water Resources

1.1.1 Providing a sound scientific base for policy making (first aim)

In order for government to develop a policy to manage EDCs in water resources, it has to be able to make rational and informed decisions. Critical information such as the **magnitude** of the pollution and the **risk** it poses to humans and animals will be essential. To provide the government with a sound scientific base to assist in developing such a policy, will be no easy task. Several problems will have to be overcome:

- **Diversity of chemicals with EDC properties**
  Although about 680 (http//ec.eu/environment/endocrine/strategy/substances.en.htm-24/11/2009) chemicals are known to have ED properties and the number is increasing rapidly, EDCs could not be classified as a single group of chemicals. Many common household products contain chemicals with ED properties, e.g. pesticides, fabric softeners, flame-retardants, preservatives, emulsifiers and pharmaceuticals. During the second phase of the research programme it was proved that these substances occur in various water resources, and humans and animals are continually exposed to them through inhalation, digestion and skin absorption (WRC Report 1402/1/08).
- **Mode of action of EDCs in the human body and in the environment**
  It is not fully understood exactly how certain EDCs influence the various hormone systems in the human body or which breakdown products are formed under different climatic conditions in the environment. This information will be essential in the development of a reliable risk assessment model. A few research projects are already in place to address this knowledge gap.
- **A human health and environmental risk assessment model** is needed to determine to what extent the level of pollution poses a threat to human health and becomes a danger to the environment. Several risk assessment models are available, but not one of them has been developed exclusively for EDCs. (In EDC research the term "Effect Evaluation" would be more appropriate and should be used instead of "Human Health Risk Assessment"). The WRC has now funded projects to address this need. Furthermore, risk assessment studies cannot be undertaken without accurate and reliable analytical data. Water systems are very dynamic and they change continuously. A programme to monitor the water resources on a continuous basis in order to obtain sufficient data is therefore necessary.

1.1.2 Development of analytical tools to acquire relevant data (second aim):

- Only limited data exist on the concentration levels of EDCs present in water resources. This is because these substances are active at very low concentrations and chemical analyses to detect such trace levels are very expensive. EDC activity in water systems may be determined by biological and biochemical methods but such analyses are no less expensive and provide only an *indication* of the activity with no further information on the identity and levels of the compound(s) that causes the activity. These methods, however, are essential tools, because chemical analysis alone cannot target all possible chemicals in a single analysis. Methods for both chemical analysis and activity testing have been developed and implemented in several laboratories at universities and other scientific organisations in South Africa (WRC Report 1402/1/08).
- During the second phase of the programme, several methods were developed and implemented to determine the mode of action of EDCs on the reproductive system of the human body as well as on the thyroid function (WRC Report 1402/1/08). In the extended plan (WRC Report KV 228/09), projects were proposed to study the effects of EDCs on other hormone systems such as the nervous system and the immune system. Some of these projects have already started.

2. METHODOLOGY

In order to reach the final goal of this initially planned 10year EDC programme, namely to develop a management programme to manage the EDC pollution problem in our water resources, the following steps need to be taken:

- Determine the current state of the science and measure the progress of the programme against the final goal
- Determine the knowledge gaps needed to reach this goal
- Determine the current capabilities and capacity so that capacity can be built and capability can be upgraded to meet the needs of the scientists
- Identify projects and develop where needed Terms of Reference (ToR) for projects for future research in this field

2.1 Determining the state of science and the needs of stakeholders

Workshops were held which were attended by stakeholders to discuss the knowledge gaps identified during the development of the extended strategic research plan (Volume 3). An urgent demand for the following items was identified:

A. **Reliable and credible data on the occurrence and magnitude of EDC pollution**
B. **Information regarding the fate and behaviour of EDCs in the environment and in the human body**
C. **Models for risk assessment**
D. **Relevant, affordable and rapid analytical methods**
E. **Facilities for analytical procedures**
F. **Guidelines for the management of EDCs in water resources**

In order to meet these demands and at the same time comply with the aims and objectives of the extended research plan of the WRC research programme, it was recommended that future research be divided into two levels and would be discussed as such in this report:

- **Fundamental research:** Research projects aimed at understanding the mode of action of EDCs, new trends in analysis and development of risk assessment models and a model to predict the environmental fate of toxic organic chemicals in the aquatic environment**.**
- **Applied research**: Field studies to determine the impact of EDCs on water resources and the environment.

Schematic presentations of the needs and the actions to be taken to satisfy these needs in order to bring the EDC research programme to a successful conclusion were included in Volume 3.

2.2 Addressing the needs and gaps – an overview

2.2.1 Needs A, D and E: Data acquisition and method development

Reliable and credible data are essential to be created during the implementation of the research plan. Data are currently still limited and not sufficient for an extensive risk assessment model.

An ongoing surveillance programme of EDCs in water needs to be developed and such a programme can only be implemented by a government department such as the Department of Water Affairs (DWA). The data generated in the surveillance programme as well as data generated in the various research projects should be stored in a Geographical Information System (GIS) and held at an institution where all researchers will have easy access to the information. The WRC was involved in the development of the National Toxicant Monitoring Programme (NTMP) of DWA and ensured that it also includes the monitoring of EDCs, but the EDC specific data that would be gathered would be very limited by the one ED activity test included in the programme at this stage.

After a specific study site for any EDC research project has been selected, data need to be acquired to determine the impact of EDCs on the environment. For this purpose the entire site should be studied, i.e. the water resource as well as the surrounding fauna and flora. The health of the human population using the water resource for drinking as well as for recreational purposes and hygiene should be included in the study. Reliable and easy-to-use analytical methods are essential for this action. Field studies as well as epidemiological studies are labour-intensive and very expensive to execute. Careful planning of such a research project is therefore of the greatest importance. Environmental modelling to predict whether a certain compound may be present in the water was investigated, but unfortunately this project was stopped due to insufficient data and loss of manpower. This should however not been neglected in future planning and identifying of projects and knowledge gaps.

Statistical analysis and the interpretation of analytical results should be approached with great care - water resources are very dynamic systems and incorrect conclusions could easily be made based on insufficient and inaccurate data.

The financial and human resources available for EDC studies are severely limited. In order to use these resources wisely, we should, at this stage, take stock of what has been achieved and where resources have to be utilised in order to complete the EDC research programme successfully.

2.2.2 Current status of data acquisition and method development

Data acquisition depends heavily on the availability of appropriate analytical methods. EDCs are active at very low levels, requiring sophisticated analytical procedures and highly trained operators.

2.2.3 Status of method development for chemical analysis

Substantial amounts of money and time were spent on the development of chemical methods and the building of capacity in research organisations. Table 1 reflects the current status of chemical analytical capability and capacity for EDC research.

Table 1: The current status of chemical analytical capability and capacity for EDC research.

EDC compounds	Current status	Capacity (Universities, para-statals and private laboratories)	Areas where more research is needed
Pesticides	Methods in place for the majority of most common pesticides	Several laboratories at accredited facilities	Methods need to be implemented for carbamates and less common pesticides
Hormones	Methods were developed for determination of the four important human female hormones	Only two laboratories, not accredited	**Urgent need for capacity building in organisations**
Alkylphenols	Methods developed and implemented	Accredited facilities available	
PCBs	Methods available	Accredited facilities available	
Dioxins and dibenzo-Furanes	Only one method available	Only one facility available	**Urgent need for capacity building in other facilities**
Flame retardants	No method available	One facility	**Urgent need for method development and capacity building**
Phthalates	Methodology well developed	Several accredited laboratories	
Pharmaceuticals	Methods available but not implemented	No facility identified	Methods need to be implemented
Veterinary drugs	Methods developed but not fully implemented	Only one laboratory not accredited	**Urgent need for facilities to implement methods**
Mycotoxins	No methods implemented	No facilities identified	Methods need to be implemented
Minerals	Methods available	Several accredited laboratories	

Note: A GC-MS-TOF (Gas chromatograph coupled with a Time-of-Flight Mass spectrometer) method was developed by the CSIR which required minimum sample preparation and with the ability to detect most classes of EDCs in one run. (Method described in Volume 2). Only very few organisations have access to a Time-of-Flight facility and the method has not been implemented.

2.2.4 Status of method development for activity testing:

a. Estrogenic activity

The Global Water Research Coalition (GWRC) recommended five tests to be used worldwide for the testing of estrogenic activity in the reproductive system. These tests are all *in vitro* tests, i.e. tests that are performed with individual cells or molecules. *In vitro* tests are sensitive, rapid and specific, compared to *in vivo* studies which are carried out on whole organisms. Most of these tests are done at universities. Table 2 reflects the current status in the country of the capacity and capability to conduct these five tests.

Table 2: Capability and capacity for estrogenic activity testing

Activity test Bioassays	**Type of test**	**Kind of test**	**Current status** (Universities, para-statals and private laboratories)	**Research needed**
Yeast screen	Recombinant yeast assay	Estrogenic activity	Three organisations have the capability but limited capacity	**Capacity needs to be built**
ER-Calux	Recombinant mammalian cell reporter gene assay	Estrogenic activity	Facilities available but too expensive to implement	Method needs to be implemented and verified
MELN	Recombinant mammalian cell reporter gene assay	Estrogenic activity	No facilities	No research needed
T47D-KBluc	Recombinant mammalian cell reporter gene assay	Estrogenic activity	One facility available	**Method needs to be implemented and verified**
E-Screen	Cellular proliferation assay	Estrogenic activity	One facility, limited capacity	**Capacity needs to be built**

b. Androgenic activity

Although the AR-Calux is similar to the ER-Calux, only a few organisations have the capability to conduct these tests.

c. Activity of the immune system and thyroid

Only one university does research on the effect of EDCs on the thyroid function. Only one facility exists for activity testing on the immune system using Interleukin 6 and an ELISA procedure.

2.2.5 Need B: Mode of action on hormone systems and fate and behaviour of EDCs in the environment.

Knowledge on the mode of action of EDCs in the human and animal body is essential in determining the risk that these substances pose to the specific organism. Table 3 reflects the

current status of the capability and capacity to conduct studies on the mode of action on hormone systems and fate and behaviour of EDCs in the environment.

Table 3: Status of the capability and capacity to conduct studies on the mode of action on hormone systems and fate and behaviour of EDCs in the environment

Hormone system	Current facilities utilised for studies	Facilities needed
Reproductive system	Eight universities and parastatal organisations	Capacity needs to be built in all facilities
Immune system	One university Limited capacity	Capacity needs to be built in other facilities
Thyroid system	One university Limited capacity	Capacity needs to be built in other facilities
Neuro system	No facilities	Facilities need to be created
Pancreas	No facilities	Facilities need to be created

2.2.6 Need C: Risk Assessment

The development of a risk assessment model to forecast the effects that EDCs may have on human health and the environment is of prime importance. Developing such a model depends on information regarding the occurrence, magnitude of contamination, cause-effect data and other parameters such as exposure pathways and lifetime exposure. Not all the information is currently available, but great progress has been made on an effects-based model.

Funds have already been allocated for the development of human and environmental health risk assessment models for EDCs. Considerable progress has been made in the human health risk assessment project (WRC Project 1749). The data generated in the monitoring programme as well as in other research projects will provide information on the **magnitude of the pollution**, while the risk assessment model should give an indication of the **risk to the population and the environment.** All this information should be helpful to the government to develop a policy that is aimed at managing the problem of EDC pollution by setting guidelines for the admissible levels of these compounds in water systems. Once the source, identity and magnitude of the pollution are known, management options and remedial actions should be decided upon.

2.2.7 Need F: Management guidelines

It became apparent that an entire set of guidelines (F) is needed to assist researchers in planning and executing EDC studies to obtain reliable and credible results that can be used with confidence in decision making. These guidelines (Appendix- initial proposals discussed at workshops) will address planning of the project, sampling and analysis as well as statistics and interpretation of results, assessment and help in recommendations to management options of resources where necessary. It will also serve as prevention of mistakes made in the past with previous projects and avoid duplication of expensive research undertaken with bad planning and expensive analysis.

The guidelines will be developed for use in catchment management monitoring of/ and prevention of EDC pollution (3.1.1).

3. SUMMARY OF PROJECTS UNDERTAKEN OR PLANNED TO MEET SAKEHOLDER NEEDS

3.1 Addressing the need for accurate and reliable data on the occurrence and magnitude of EDCs in South African water resources

All the stakeholders expressed a need for accurate, reliable and repeatable analytical results. No research study or management action is possible without reliable analytical data. Accurate and reliable analytical data can only be generated in reliable (preferably accredited) facilities using validated methods. In South Africa such facilities are limited - accreditation is very expensive and the requirements very strict. Universities, for example, cannot be accredited as they cannot prove continuity of staff when students are used for routine work. The need for a set of guidelines on planning and execution of EDC studies, including analytical procedures used for chemical analysis and activity testing, was recognised by the management team of the WRC Research Programme on EDCs. The WRC subsequently decided to fund the compilation of a manual to provide guidelines to researchers, education institutions, industry and managers in planning, managing and assessment of EDC studies.

3.1.1 Compilation of a manual of guidelines for planning, management and assessment of studies on EDCs in water resources (Fundamental Research)

A workshop was held in Stellenbosch during June 2007 where the format of such a manual was discussed. It was decided to divide the manual into four different volumes:

Volume 1: Monitoring and assessment (Planning of EDC study)
Volume 2: Sampling guide
Volume 3: Toolkit/Methods
Volume 4: Management options

A project leader was appointed for each volume and a team of experts selected. The project leader and the team of experts developed the content of each volume. Details of the initially proposed content of the manual may be found in APPENDIX 1.

3.1.2 Current status of research projects undertaken as part of the WRC's EDC Research Programme

More information about the current status of research projects funded and supported by the WRC appears in Table 4.

Table 4: Status of Research Projects: Completed/Ongoing/Planned According to Overall Aim of this Project

Aim	WRC Project no.	Project Title	Research Undertaken by	Project Status or Term	Reporting Status
Strategic Planning and Management	K8/447	WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 1. Strategic Research Plan for Endocrine Disrupters in South African Water Systems	AEC Burger (Private Consultant)	Completed	WRC Report KV 143/05
	K8/650	WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 3. Extended Strategic Plan for the Endocrine Disruptor Research Programme of the WRC, 2006 – 2010	AEC Burger (Private Consultant)	Completed	WRC Report KV 228/09
	K8/760	WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 4. Implementation of an Extended Strategic Research Plan on Endocrine Disruptor Chemicals in Water Resources (**this report**)	AEC Burger (Private Consultant)	Completed	This report
	1816	Guidelines for EDC management in water resources. Volume 3: Bio-assay toolbox	University of Pretoria	Ongoing 2008-2010	
	1853	Quality control and assurance guideline for South African toxicity testing laboratories	University of Pretoria	Ongoing 2008-2010	
	1915	Guidelines for EDC management in water resources. Volume 1: Monitoring and assessment guide (Planning of an EDC study)	University of Pretoria	Ongoing 2009-2012	
	1933	Guidelines for EDC management in water resources. Volume 4: Management options for EDCs	Golder Associates Africa (Pty) Ltd	Ongoing 2009-2012	
	1983	Guidelines for EDC management in water resources. Volume 2: Sampling procedures for EDCs and toxins.	Golder Associates Africa (Pty) Ltd	2010-2012	
Data Acquisition and Analytical Method Development	1402 (including 1469-1475 and 1555 below)	WRC Programme on Endocrine Disrupting Compounds (EDCs) Volume 2. Implementation of a Research Programme for Investigating Endocrine Disrupting Contaminants in South African Water Systems	AEC Burger (Private Consultant)	Completed	WRC Report 1402/1/08

Table 4: Status of Research Projects: Completed/Ongoing/Planned According to Aim (Continued)

Aim	WRC Project no.	Project Title	Research Undertaken by	Project Status or Term	Reporting Status
	1470	Sampling and Analysis for Selected EDCs in Selected Water Environments in the Makhatini Flats (Jozini Dam), Hartbeespoort Dam, Vaal River Barrage and Jukskei River (Rietvlei Dam)	Agricultural Research Council	Completed	Included in WRC Report 1402/1/08
	K8/1471	Literature Review: Sex Determination and Gonad Differentiation Control Pathways in Fish as Bio-indicators for Endocrine Disruption in Aquatic Systems	University of Stellenbosch	Completed	Included in WRC Report 1402/1/08
Data Acquisition and Analytical Method Development	1532	An Investigation of the Estrogenic Activity in Water from Selected Drinking Water Treatment Processes	CSIR	Completed	WRC Report 1532/1/08
	1555	An Investigation into the Occurrence of Steroidal Hormones (Estrogens) in Sewage Effluents using Biological, Biochemical and Chemical Techniques	CSIR Environmentek	Completed	Included in WRC Report 1402/1/08
	1674	Environmental Exposure and Health Risk Assessment in an Area Where Ongoing DDT Spraying Occurs	University of Pretoria	Completed	In press
	1686	Endocrine disruptive chemical (EDC) activity and health effects of identified veterinary compounds in surface water and groundwater	School of Health Systems and Public Health, University of Pretoria	Ongoing 2006-2010	In press
	1774	Scoping Study to Determine the Potential Impact of Agricultural Chemicals (Pesticides) on Water Resources of South Africa	Burger and Nel (Private Consultants)	Completed	WRC Report 1774/1/08
	K8/474	The Chemical, Biological and Immunological Evaluation of Selected Estrogens found in Sewage Effluent in the GaRankuwa/CRC Area	Medical University of Southern Africa	Completed	Included in WRC Report 1402/1/08
	K8/480	Sampling and Chemical Analysis of Water from the Josini Dam for EDC Pesticides	Agricultural Research Council	Completed	Included in WRC Report 1402/1/08
	consultancy	Determination of Estrogens in Water and Sediment	AMPATH	Completed	Included in WRC Report 1402/1/08

Table 4: Status of Research Projects: Completed/Ongoing/Planned According to Aim (Continued)

Aim	WRC Project no.	Project Title	Research Undertaken by	Project Status or Term	Reporting Status
Data Acquisition and Analytical Method Development	K8/479	Literature Review: Trace Level Determination of Selected Pesticides, PCBs and Alkylphenols in Water and Sediment by GC-Time-of-Flight MS	CSIR Bio/Chemtek	Completed	Included in WRC Report 1402/1/08
	K8/476	Sampling and Analysis for the Presence of EDCs in Surface Waters and Sediments in the Venda Region	Pretoria Technikon	Completed	Included in WRC Report 1402/1/08
	K8/472	Development of *in vitro* Laboratory Screening Methods for EDC Activity (Hazard Identification)	University of Pretoria	Completed	Included in WRC Report 1402/1/08
	1473	Development of Biomarkers in Selected Sentinel Species as Indicators of EDC Exposure	University of Pretoria	Completed	Included in WRC Report 1402/1/08
	1471	Water Resource Monitoring and Method Development and Validation of Biochemical Methods to Determine Endocrine Disrupting Activity in Water Systems	University of Stellenbosch	Completed	Included in WRC Report 1402/1/08
	K8/471	Development of Biomarkers for Determining EDC Contamination in Surface Water	University of Stellenbosch	Completed	Included in WRC Report 1402/1/08
	K8/478 and 1472	Method Development for Biochemical Procedures Related to Estrogen and Androgen Screening of Water and Sediment Samples	CSIR Environmentek	Completed	Included in WRC Report 1402/1/08
	1505	The Use of Sentinel Species to Determine the Endocrine Disruptive Activity in an Urban Nature Reserve	University of Pretoria	Completed	WRC Report 1505/1/07
	1561	Persistent organic pollutants (POPs) in the environment	North West University	Ongoing	
	1590	The Estrogenicity of Sewage Effluent Entering the Eerste-Kuils River Catchment System	University of the Western Cape	Completed	WRC Report 1590/1/08
	1557	An investigation into the occurrence of EDCs, organochlorine pesticides and heavy metals) in surface waters in the Northern Province	University of Venda	Suspended	

Table 4: Status of Research Projects: Completed/Ongoing/Planned According to Aim (Continued)

Aim	WRC Project no.	Project Title	Research Undertaken by	Project Status or Term	Reporting Status
Data Acquisition and Analytical Method Development	1475	The Application of the *Chemprop* (Chemical Property Estimation System) Model for South African Conditions to Predict the Environmental Fate of Toxic Organic Chemicals in the Aquatic Environment	Rand Water in association with UFZ (Umweltforschungzentrum), Germany	Suspended	1475
	926 and 1253	The development and validation of bioassays to detect estrogenic and anti-androgenic activity using selected wildlife species	University of Stellenbosch	Completed	WRC Reports 926 and 1253/1/05
	K8/828	Rapid enzymatic detection of organo-chlorine pesticides in water	Rhodes University	Ongoing	
	1534 Continued with K8/882	New Detection Methods for EDCs. Continued as: Immobilisation of the thyroid receptor on inert membrane contactors for the rapid detection of EDCs	University of Stellenbosch	Ongoing	Report to be published will cover both projects
	1706	Environmental assessment in an area where ongoing DDT spraying occurs	Rhodes University	Ongoing 2007-2010	
	1756	Investigation into the effects of water quality (organic vs. inorganic) on the immune systems of humans	University of the Western Cape	Ongoing 2007-2010	
	1883 not EDC specific	Assessment of the prevalence of organic compounds in raw and treated water for potable purposes, their fate in current treatment plants, and compilation of a guideline on best available technology for the removal thereof	University of Johannesburg	Ongoing 2009-2012	
	1902	Rapid enzymatic detection of organochlorine pesticides in water	Rhodes University	Ongoing 2009-2011	
	1816	Guidelines for EDC management in water resources. Volume 3: Bio-assay toolbox	University of Pretoria	Ongoing 2008-2010	
	1932	A study of the interactive effects of pesticide mixtures in water on selected species	University of Stellenbosch	Ongoing 2009-2012	
	1956	Investigation of the contamination of water resources by agricultural chemicals and the impact on environmental health	CSIR	2010–2015	

Table 4: Status of Research Projects: Completed/Ongoing/Planned According to Aim (Continued)

Data Acquisition and Analytical Method Development	K8/872	Development of guidelines for chemical analysis of endocrine disruptor chemicals (EDCs) in water resources of South Africa for inclusion in volume 4 of the EDC guidelines	AEC Burger (Private Consultant)	Completed	Incorporated in Vol 3 Guideline
	K8/920	The finalization of a laboratory manual with selected bio-assays for the detection of estrogenic activity in water.	University of Pretoria	Ongoing	
	K8/850	Scoping study on the sources and occurrence of common brominated flame retardants in South Africa.	Tshwane University of Technology	Ongoing	
Developing a Risk Assessment Model	1680	Thyroid-disrupting Activity in South African Waters. Amphibian Metamorphosis as Biological Model to Study Effects of EDCs on Thyroid Function	Dept of Zoology, University of Stellenbosch	Ongoing	
	K8/648	Health Risk Assessment Protocol for Endocrine Disrupting Chemicals	CSIR	Completed	KV206/08
	1712	Development of an ecosystem risk assessment model to determine the risk of EDCs in the water environment	University of Stellenbosch	Ongoing	
	1749	Development and testing of a health risk assessment framework to derive guidelines for endocrine disruptors (EDCs) in drinking water	CSIR	Completed	1749/1/09

3.2 Addressing the need for analytical facilities

There is still a lack of analytical facilities in the country. For international recognition of results and to ensure that all decisions taken in the management of EDCs are based on correct and reliable analytical results, accredited local laboratory facilities are required. Only a few such facilities are currently available. EDCs are active at extremely low levels and therefore specialised and very sensitive methods are required to determine these substances at the relevant concentrations. Operating an ultra-trace laboratory not only requires very sophisticated instrumentation, but also highly qualified operators with years of experience in this field. EDC field studies also require the analysis of a large number of samples to make the results statistically valid. Only a few selected laboratories in South Africa have the capacity to analyse such large numbers of samples taken from different matrices. An effort should be made to create and/or build such facilities. During the execution of the different projects in the EDC programme, a total of forty-two students were trained at different educational institutions. Only two of them remained - their main reasons for leaving were poor remuneration and a shortage of permanent appointments.

Three scenarios are proposed to address this problem:

Scenario 1: Create a national facility which will be able to perform all types of water-related analysis, similar to the EPA laboratory in the USA or the RIVM in The Netherlands

Scenario 2: Update and upgrade the current facilities at parastatal, governmental and private laboratories to enable them to undertake chemical analyses to determine most of the critical EDCs and subcontract the more complex analyses to overseas laboratories

Scenario 3: Create "Virtual Laboratories", i.e. centres of excellence at selected universities for biochemical/biological analysis and commercialise them to operate as private facilities so that they may employ full-time staff and obtain accreditation.

4. CONCLUSIONS

- **Progress** has been made in 2006-2010 to meet the extended aims of the programme.
- **The occurrence of EDCs** in water, sediment and biological tissue of aquatic and terrestrial animals was confirmed.
- It was established that agricultural practice, industrial and mining activity, human excreta and other natural occurrences were the main **sources of contamination**.
- **Detrimental hormonal effects of EDCs were observed** in aquatic and terrestrial animals as well as in humans. Although terrestrial animals and humans are exposed to EDCs from other sources such as food and personal care products, aquatic animals are only exposed through water. Studies concentrated mainly on the reproductive system, but some researchers found indications that the thyroid and immune system were also affected.

- **The actual magnitude of EDC** contamination is not yet known and the full impact on the environment can therefore not yet be established.
- **Risk assessment** depends on information regarding occurrence, magnitude of contamination, cause-effect data and other parameters such as exposure pathways and lifetime exposure. Not all the information is currently available. This should, however not prevent the programme from moving into the final stages of the extended research plan, i.e. management and remedial action. Several of the projects will continue in 2010, while themes for follow-up projects have been identified after consultation with all interested parties to meet the needs of the various stakeholders.

5. RECOMMENDATIONS

a. Analytical needs

During the "needs and gaps" analysis, it became clear that the need for accurate, reliable and repeatable analytical results formed the top priority for all stakeholders, especially in the field of EDC activity testing (biological/biochemical analysis). It is therefore of the utmost importance that methods used locally for activity testing be standardised and harmonised with methods used in other countries. The contact and cooperation with the Global Water Research Coalition (GWRC) should therefore continue and laboratories active in this field should be encouraged to take part in inter-laboratory tests. Processing of results obtained from these biological/biochemical tests needs to be done with caution, since different ways of calculation and interpretation may lead to contradictory conclusions. The issue of calculation worldwide is still a matter of contention. Workshops held in the past in South Africa on this subject did not provide clear guidelines. It is therefore essential that a workshop should be held to clear up this issue. The methodology and calculation procedures selected at such a workshop should then be included in the manual of guidelines on EDC studies (Volume 3). Standardised methods for target (chemical) analysis should be continually reviewed and new methods included in this manual. All EDC studies require chemical analyses to determine very low levels of EDCs in different matrices which, in its turn, require highly sensitive methods and sophisticated instrumentation such as GC-MS and LC-MS. The search for and development of rapid and cost effective analytical procedures, both activity testing procedures and target analysis, should therefore continue.

b. Impact assessment:

The water matrix is very complex and EDCs from various polluting sources (agricultural practice, industrial and mining activity and human excreta) may be present in a water body at a specific moment. Because of the number of variables, it is virtually impossible to determine the impact of a certain group of pesticides on a water resource during a field study. Activity testing gives an indication of *total* EDC pollution, but provides no indication of which specific EDC or group of EDCs is

present. A number of EDCs may be determined by chemical analysis using multi-residue methods, but currently there are more than 680 chemicals suspected of ED activity and the cost for determining each individual EDC would obviously be astronomical. A laboratory study is recommended to determine the potential impact of a *group* of chemicals suspected to be in the water. The following protocol has been proposed and used for the determination of e.g. pesticides in an area:

1. Select of a study site using the selection criteria formulated during the planning of the study (it may be a Water Management Area, a river and/or a dam).
2. Determine certain physical properties of the water resource, e.g. pH, average temperature, dissolved oxygen, mineral content, suspended matter, time of day and time of year.
3. Identify the crops grown and animals held in the area by using Table 1 of the report: *Scoping Study on Potential Impact of Agricultural Chemicals on Water Resources* (WRC Report 1774/1/08).
4. Identify the agricultural chemicals used on the selected crops, again referring to Table 3 of the abovementioned report.
5. From this information, combined with the physical data of the study site, it may be possible to determine the pesticides most likely used in that region
6. Prepare mixtures of the various pesticides identified. It suggested that these "cocktails" should be made up from different classes of pesticides, i.e. fungicides, insecticides and/or herbicides.
7. Create a synthetic water resource in the laboratory, using distilled water to which the minerals found naturally in the water resource are added in the relevant quantities. This is necessary because the mineral content of the water may influence the solubility of certain pesticides. A control study in pure distilled water needs to be run concurrently with the study in the synthetic water mixture.
8. Add different concentrations of the "cocktails" to both the synthetic and distilled water.
9. Expose a variety of aquatic animals such as fish, tadpoles or other selected test animals as well as small mammals to the different concentrations of the mixtures and determine any detrimental effects that the EDCs may have on these organisms.
10. Use bio-assays (*in vitro* and *in vivo)* to determine EDC activity in the water.

Expected Outcome:

The effect that the various mixtures of selected agricultural chemicals may have on the hormonal systems of test specimens will be determined **without interference of other EDCs**. Therefore, the results from these tests may be used as a point of departure in risk assessment studies to predict the possible impact of a specific compound on a water resource.

The same protocol may be applied for industrial chemicals and pharmaceuticals.

c. Remedial action

The WRC funded studies to investigate the *removal* of EDCs in drinking water (DWTP) and sewage water treatment plans. Results indicated that the majority of EDCs is removed in the large, sophisticated DWTPs resulting in very low residues of EDCs remaining in drinking water supplied by these systems. Smaller DWTP are unfortunately, less effective in removing EDCs. Low levels of hormones and certain pharmaceuticals were detected in the water after purification in smaller DWTP. The female hormone, 17β-estradiol and its breakdown products estrone and estriol as well as the synthetic hormone ethinylestradiol (used as contraceptive) show ED effects at very low levels (0.3ng/ℓ). When water is circulated continuously, there may be a build-up of these hormones in drinking water. Currently no data exist on the levels of hormones present in drinking (tap) water. These hormones, as well as pesticides and industrial chemicals with EDC properties, were detected in rivers and dams across the country and could be fromnon-compliance of sewage works to effluent standards and from informal settlements next to water resources. Although the full impact of these chemicals on the environment has not been established yet, some detrimental effects on the hormonal systems of aquatic and terrestrial animals have already been observed. Humans are not only exposed to drinking water, but also to surface and ground water. It is therefore important to focus on *all* water systems in any study aimed at formulating recommendations for remedial action.

While the supply of drinking water to all citizens of the country has been one of the pillars of government policy, the *quality* of the drinking water supplied to communities should also become more of a priority. The upgrading of water purification systems will have severe cost implications and any decisions made in this regard should be carefully considered. A wide spectrum of stakeholders, engineers and scientists as well as community leaders should be part of the decision-making and the implementation of any new or altered systems.

d. Knowledge dissemination

A substantial number of research projects on EDCs were undertaken at various institutions and universities during the last ten years. Despite the call by the WRC for a coordinated approach, there is also the research done and ongoing by post-graduate students at universities outside the EDC projects of the WRC. It is therefore recommended that a website, containing summaries and conclusions of all these projects, should be developed to ensure that scientists working in this field have access to the information.

e. Future of the WRC EDC Research Programme

It is recommended that the WRC's programme on EDC research should continue, but that the focus should now be more on the *management* of the country's water resources, but the innovative scientific research should not be neglected in seeking more and better understanding on EDCs and its effects. The WRC has taken the lead in supporting ground-breaking research on the subject of pollution by EDCs in South Africa. It is

hoped that the WRC will continue with the EDC programme to ensure that the environment and generations to come may have access to safe and clean water.

APPENDIX 1

GUIDELINE MANUAL

Volume 1: Monitoring and Assessment Guide (Planning of an EDC Study)

Project leader: Prof. James Meyer

Scope:

This volume should provide background and definitions as well as key issues related to planning and executing an EDC study.

- Define (a) monitoring and (b) assessment
- Definitions: water resource, surface water, ground water etc.
- Determine status of water resource (depending on target audience)
- Evaluate costing and costing concerns
- Define key questions: short-term assessment (grab samples) vs. long-term monitoring (ecology, monitor sentinel species)
- Define monitoring needs regarding frequency of sampling, time of sampling and sampling sites
- Interpretation/integration of results. Storage of results and statistics.
- Roadmap – decision making/management intervention (when to start managing)
- International trends review

Contents:

- General introduction (refer to mode of action – EDC definition)
- List of EDCs:
  Current: Estrogenic, androgenic, anti-androgenic
  Future: Thyroidogenic, other steroidogenic.
- Planning of an EDC study: explain importance of quality control and good laboratory practice (GLP), reason for study, defining of key issues (short-term assessment vs. long-term monitoring), selection of study site, selection of EDCs, selection of analytical methods, interpretation of results, costing concerns, communication of information. Cross reference to Volumes 2, 3 and 4
- Chemical analytical methods and bioassays. Cross reference to Volume 3
- Occurrence, fate and behaviour of EDCs
- Evaluation of results and risk assessment (to the ecology, humans or the combination of the two). Cross reference to Volume 4
- Link to clinical context (bio-indicators)
- Treatment/mitigation/remediation (allocation of funds and resources where necessary). Cross reference to Volume 4
- Communication process – collaboration with health effect groups/researchers. Cross reference to Volume 4
- Information on cause and effect (where and/or what is available for remediation). Cross reference to Volume 4
- Road map for management intervention
- International trends review

Volume 2: Sampling Procedures for EDCs and Toxins

Project leader: **Dr Ralph Heath**

Scope:

This chapter will only address the taking of samples, transport and storage of samples up to delivering the samples to the relevant laboratory.

- Defining the purpose of the monitoring/assessment study
- Determining the kind of sample needed to be taken to address the specific pollution problem
- Describing the method of sampling for different matrices (grab sample, composite sample, continuous sampling)
- Describing the equipment needed for different sampling procedures including pre-treatment of glass and plastic equipment prior to sampling
- Describing the method of storage and transport to laboratory (including time from sampling to laboratory analysis and preservation of samples during storage and transport)
- Development of field observation form (location, sampler, date, sampling method, time of day, temperature, pH of water, dissolved oxygen, external events such as pesticide spraying, fish deaths, time taken to transport samples to laboratory)
- Supply list of laboratories for relevant tests
- Problems/trouble shooting
- Safety precautions
- Community involvement

Contents:

- Introduction to chapter describing the principles of good laboratory practice (GLP) and the importance of applying these principles to the sampling protocol
- Description of GLP protocol as relevant to the type of study, the specific matrix to be sampled, equipment needed, maintenance and calibration of equipment as well as the storage and transport of samples. (See scope above)
- Examples of GLP documentation for domestic and field sampling
- List of criteria for relevant analytical laboratories capable of analysing specific samples
- Description of methods for field extraction of samples, their storage and transport conditions
- Training of samplers
- Problems and trouble shooting
- Safety precautions
- Handling community communication issues

Volume 3: Bio-assay Toolbox
Project leader: Prof. Tiaan de Jager

Scope:
This volume will deal with the process from the time that samples are delivered to a laboratory until results are submitted to the person/institution/project leader that submitted the samples for analysis

- Water quality measurements such as pH, conductivity, dissolved oxygen, etc.
- Technical documentation (refer to GLP document in Volume 2)
- Description of different water sources and which protocol should be used for each type of study
- Description of difference between activity testing (bio-assays) and chemical analysis, the purpose of each and the limitations of each. Selection of appropriate methods for specific study
- Protocols of different methods:
  - Bio-assays:
  - Current:
  - Estrogen-related
  - Androgen-related
  - Thyroid-related
  - For future:
  - Immune system
  - Neurological system
  - Metabolic modulation
- Chemical analysis
- The importance of accreditation and proficiency testing.
- Current list of EDCs (updateable)
- Criteria for new methods to be added as new EDC compounds are added to list
- For each method described, the following should be addressed:
  - Introduction to strains and guidelines (bio-assays)
  - Test environment (temperature control, sterile cabinets etc.)
  - Equipment needed (instrumentation, glassware and consumables)
  - Technical skills of analysts
  - Safety precautions
  - Chemicals needed (including suppliers of critical chemicals and standards)
  - Test procedure (protocol)
  - Limits of detection
  - Quality controls (negative and positive controls, blanks and recovery tests)
  - Method of calculation of results (standard curves)
  - Possible interference
  - Ethical considerations
  - Cost per unit
  - Duration (turnaround time)
  - Capacity (size of batch)

Contents:

- Introduction describing the importance of quality control in the laboratory, documentation and safety measures
- Documents for receipt of samples: state of samples, test(s) required, date of delivery, names of persons who delivered and received the samples, name and address of institution requiring analysis, identity number of samples, etc.
- Quality control documentation for handling and analysing samples in the laboratory
- Storage and treatment of samples in the laboratory
- Analytical procedures:

  This section should be divided into the following sub-sections:

  1. Water quality measurements: pH, total dissolved oxygen, conductivity, etc.
  2. Biological/biochemical methods (activity measurement)
     In vitro methods
     In vivo methods
  3. Chemical analysis (quantitative measurement)
     Organic chemical analysis
     Inorganic chemical analysis
     ELISA methods
- Each method should describe the following:

  Introduction to strains and guidelines (bio-assays)
  Test environment (temperature control, sterile cabinets etc.)
  Equipment needed (instrumentation, glassware and consumables)
  Technical skills of analysts
  Safety precautions
  Chemicals needed (including suppliers of critical chemicals and standards)
  Test procedure (protocol)
  Limits of detection
  Quality controls (negative and positive controls, blanks and recovery tests)
  Method of calculation of results (standard curves)
  Possible interference
  Ethical considerations

Volume 4: Management Options for EDCs

Project leader: Dr R Heath

Scope:

This volume will deal with the issues after results have been submitted to the relevant institution/project leader/organisation.

- Risk assessment/evaluation
- Statistics on relevant study sites
- Target of remedial action: drinking water/environmental protection
- Treatment/mitigation options

Contents:

- An introduction, highlighting the importance of reliable analytical results, sensible statistical analysis, problem evaluation and risk assessment for management of polluted areas. Give an indication of possible remedial/mitigation options for different situations.
- Situation evaluation and risk assessment. This will include the possible impact of the specific EDCs on humans and wildlife, the determination of the different sources of the EDC pollution and the impact of each source on a specific study site. The different pathways of exposure and how EDCs react on receptors should be explained.
- Treatment/mitigation options available for the various areas and specific pollution problems. This will include the target of remedial action (drinking water/environmental protection)
- Communication with communities and other stakeholders (such as farmers, businesses, and government departments) during the study and also during the remedial action phase.