Endocrine-Disrupting Compounds - Sampling Guide

Volume II

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Report to the

Water Research Commission

by

Golder Associates Africa

WRC Report No. TT 561/13

May 2013

Obtainable from

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The publication of this EDC Guideline Volume series (comprising five volumes) emanates from a study commissioned and funded by the Water Research Commission entitled: "Development of a Sampling Guide, Volume 2, of the Manual of Guidelines for the Management of EDCs in water resources" (WRC Project No. K5/1983).

The templates appearing in the appendices are also available on disc to allow them to be updated or modified for different contexts.

ISBN 978-1-4312-0422-9
Printed in the Republic of South Africa

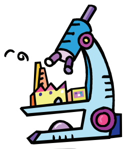
EDC GUIDELINE VOLUMES

The primary purpose of these guideline volumes is to facilitate appropriate water resource management in South Africa relating to endocrine disrupting compounds (EDCs). They present a consolidation of EDC research, current knowledge and best practices.

Volume I: Introduction

Volume II: Sampling Guide

Volume III: Bioassay Toolkit



Volume IV: Monitoring and Assessment

Volume V: EDC Management in Catchments

ACKNOWLEDGEMENTS

The authors would like to thank the Reference Group of the WRC Project for the assistance and the constructive discussions during the duration of the project:

Dr K Murray	Water Research Commission (Chairperson)
Mr C Moseki	Water Research Commission
Dr S Jooste	Department of Water Affairs
Prof C de Jager	University of Pretoria
Dr J Meyer	University of Pretoria
Prof N Casey	University of Pretoria
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The funding of the project by the Water Research Commission and the contributions made by the members of the Reference Group are gratefully acknowledged.

EXECUTIVE SUMMARY

EDCs represent a diverse range of man-made chemicals that may be discharged into the environment from many sources and have been detected in people and wildlife as well as in environmental samples. Some of these are persistent in the environment while others are not. These chemicals either mimic or antagonise the function of hormones. They may interact with physiological systems and cause alterations in development, growth and reproduction in wildlife and particularly in exposed fish.

The objective of this sampling guide is to provide guidance on the sampling, preservation, transport and storage of water, sediment, biological and air samples to be analysed for EDCs.

This Sampling Guide contains a brief background to EDCs, rationale for sampling EDCs in the environment, and which EDCs to sample for. A summary is given of the assessment process required to design a sampling programme, and details are provided on the importance of incorporating quality assurance (QA) and quality control (QC) procedures in a sampling programme. The different types of QA/QC samples for water, air, sediments and tissues (biological) are described.

The procedures that should be followed when sampling water, sediment, tissues (biological) and air are outlined. For each of these matrices, details on pre-field preparation, site selection, sample collection, transport, storage and analysis and examples of field data sheets to be used during the sampling exercise are provided.

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ABBREVIATIONS

ACE	activated carbon extraction
ADI	allowable daily intake
ECD	electron capture detector
EDC	endocrine-disrupting chemical
EDTA	ethylenediamine tetraacetic acid
FID	flame ionisation detector
FLD	fluorescence detector
GC	gas chromatograph
LC	liquid chromatograph
LLE	liquid-liquid extraction
LOD	limits of detection
MS	mass spectrometer
NOEL	no-observed-effect-level
PCB	polychloronated biphenyl
POP	persistent organic pollutant
QA	quality assurance
QC	quality control
SOE	Soxhlet extraction
SPE	solid phase extraction
WRC	Water Research Commission

GLOSSARY

Active air sampling	Sampling which requires electrical supply.
Analyte	A compound/substance tested for by a specific method.
Contaminated sediment	Sediment that contains chemical substances at concentrations that could potentially harm sediment dwelling organisms, wildlife, or human health.
Endocrine disrupting chemical (EDC)	Also referred to as endocrine disrupting compounds, EDCs are chemical substances that are not produced by the body; however, they mimic or antagonise natural hormones.
Field blank	An aliquot of reagent water or other reference matrix that is placed in a sample container in the field, and treated as a sample in all respects, including exposure to sampling site conditions, storage, preservation, and all analytical procedures.
Fungicide	A pesticide compound specifically used to kill or control the growth of fungi.
Inorganic	Composed of chemical compounds that do not contain carbon as the principal element (excepting carbonates, cyanides and cyanates). Matter other than plant or animal.
Herbicide	A chemical pesticide designed to control or destroy plants, weeds, grasses or algae.
Insecticide	A pesticide compound specifically used to kill, repel or control insects.
Maximum holding time	The length of time between preservation of a collected sample and preparation for analysis.
Method detection limit	Method detection limit is the lowest concentration of an analytical parameter in a sample that can be detected, but not necessarily quantified.

No-observed-effect-level (NOEL)	The highest dose in an appropriate study that is not associated with the adverse effects on the test organism**.**
Organic	Composed of chemical compounds based on carbon chains or rings and also containing hydrogen with or without oxygen, nitrogen or other elements.
Passive air sampling	Sampling without the need for electrical power.
Pesticide	Substances or mixtures of substances intended (i) for preventing, destroying, repelling or mitigating any pest or (ii) for use as a plant regulator, defoliant or desiccant.
Quality assurance	The implementation of all activities that minimise the possibility of quality problems occurring. These include, among others, training, instrument calibration and servicing, quality control, producing clear and comprehensive documentation.
Quality control	The process of ensuring that recommended monitoring procedures are followed correctly by detecting and correcting quality problems when they arise, so that the accuracy of primary observations or measurements is (a) defined; (b) within acceptable limits; and (c) recorded (DWAF, 2005b).
Reagent water	Water demonstrated to be free from analytes of interest and potentially interfering substances at the method detection limit for the analyte.
Sampling site	General area from which samples are to be taken.
Sediments	Inorganic and organic material that is suspended in and being transported by surface water or that has settled out and been deposited under surface waters.
Tissue	A group of cells, along with the associated intercellular substances, which perform the same function within a multicellular organism.

CHAPTER 1

INTRODUCTION

Background

Endocrine disrupting compounds (EDCs) are defined as chemical substances or a mixture of substances that are either natural or synthetic and have the capacity to alter function(s) of the endocrine system and consequently cause adverse health effects in organisms. They can cause disruptive effects at exposure levels up to a million times lower than carcinogen exposure levels of concern. Before 1999 only limited research had been done on possible contamination of EDCs in South Africa (Burger, 2008). This resulted in growing concerns, public debates and lack of consensus among scientists regarding the potential effects of a number of chemicals that have the potential to alter the normal functioning of the endocrine system (WRC Technical Brief, 11 June 2009).

This document is one in a series of five EDC guideline volumes. The decision to develop these guidelines was taken by the Water Research Commission (WRC) at a strategic planning workshop held in Stellenbosch during June 2007. A need was identified to provide guidelines to stakeholders, government managers and policy-makers with information on EDC management as well as background and scientific information on the EDC phenomena.

Rationale

The past two decades have witnessed growing scientific concerns and public debate over the potential adverse effects that may result from exposure to EDCs. Concerns regarding exposure to these EDCs are due primarily to:

- Adverse effects observed in some wildlife, fish, and ecosystems.
- The increased incidence of certain endocrine-related human diseases.
- Endocrine disruption resulting from exposure to specific environmental chemicals observed in experiments using laboratory animals.

These concerns have stimulated many national governments, international organisations, scientific societies, the chemical industry, and public interest groups to establish research programmes, organise conferences and workshops, and form expert groups and committees to address and evaluate EDC related issues.

Which EDCs to sample for

Considerable research has now been undertaken in relation to EDCs in South Africa. However, we have barely seen the tip of the iceberg and there are still far more unknowns than knowns. South African legislation allows for research, assessment and monitoring as well as implementation of specific actions related to minimising impacts from EDCs on both the environment and human health. More details on the legislative aspects are available in **Volume I: Introduction**.

A list of EDC substances has been compiled, taking into consideration their usage in South Africa (present and past). This was supplemented by listing compounds which may be important to South Africa's main trading partners (Burger, 2005). Table 1 lists the organic EDCs. Table 2 provides a comprehensive list of inorganic constituents necessary for detailed risk assessments. Some of these constituents have endocrine disrupting properties

Table 1: List of priority EDCs (Burger, 2005)

Insecticides	Herbicides	Fungicides	PCBs	Alkylphenols	Veterinary drugs	Plasticisers	Fire retardants	Others
Organochorines DDT, DDE, DDD Dieldrin, Aldrin Endrin, α-Endosulfan β-Endosulfan Endosulfan-sulphate Heptachlor Heptachlor epoxide Lindane (γ-BHC) ***Organophosphates*** Azinphos-methyl Chlorpyrifos Parathion ***Pyrethroids*** Cypermethrin Deltamethrin ***Carbamates*** Aldicarb Carbaryl	2,4-D Acetochlor Alachlor Amitrole Atrazine Diuron Linuron Metribuzin Propazine Simazine Terbuthylazine	Benomyl Carbendazim Cyhexatin Fentin hydroxide Metiram Procymidone Vinclozolin	Polychlorinated biphenyls	*p*-Nonylphenol l Nonylphenol ethoxylates *p*-Octylphenol Octylphenol ethoxylates	***Synthetic hormones*** Diethylstilbestrol (DES) Ethinyl estradiol 17β-Estradiol ***Androgenic steroids*** Trenbolone Nortestosterone Methyltestosterone ***Estrogenic action*** Zeranol ***Natural hormones*** 17α-Estradiol Testosterone Progesterone ***Beta agonists*** Clenbutarol Salbutanol Mabutanol Zilpaterol **Hormones** 17β-Estradiol Oestriol Oestrone 17α-Ethinylestradiol	Bisphenol A Dicyclohexyl phthalate Diethyl phthalate Diethylhexyl adipate Dihexyl phthalate Dipentyl phthalate Dipropyl phthalate	Polybromobiphenyl ethers	Benzo(a) perene n-butyl benzene Styrene Dichlorophenol *(dye intermediate)* Benzophenone *(raw material in medical products)* 4-nitrotoluene *(2,4-dinitrotoluene intermediate)* Octachlorostyrene *(by-product of organochlorine compounds)*

Table 2: Inorganic water quality constituents recommended for detailed risk assessments (Meyer et al., 2012)

Inorganic macro-elements relevant to hazard and risk assessment for recognised water uses	
Anions: Fluoride Nitrite Nitrate Chloride Sulphate Carbonate Phosphate Bicarbonate	**Cations:** Sodium Potassium Calcium Magnesium Boron
Additional parameters calculated or included: Total dissolved solids, electrical conductivity and pH Alkalinity, sodium bicarbonate, and sodium carbonate Permanent hardness and temporary hardness	
Inorganic trace elements relevant to hazard and risk assessment for recognised water uses	
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	

Target audience

This Sampling Guide is intended for all individuals who regulate or manage natural resources, especially water, land uses that impact on water resources; human health, as well as domestic animal and wildlife health, bulk supplies of safe drinking water, and those who inform the above regulators and managers.

Objectives

The objective of this sampling guide is to provide guidance on sampling, preservation, transport and storage of water, sediment, tissue (biological) and air samples to be analysed for EDCs. Guidance is also provided on how to select a correct sampling programme. The importance of incorporating quality assurance and quality control (QA/QC) procedures within the sampling programme is also outlined.

Structure of the guideline

The structure of this guideline is set out below

Chapter 1: Introduction

This chapter contains a brief background to EDCs; rationale for sampling EDCs in the environment; which EDCs to sample for; the target audience; the objectives; the structure of the document; and analysis categories.

Chapter 2: Sampling programme design

General guidance on sampling design is given in this chapter, as well as a summary of the assessment process required to design and initiate the sampling programme.

Chapter 3: Quality assurance and quality control

An account is given of the importance of incorporating quality assurance (QA) and quality control procedures (QC) in a sampling programme and different types of QA/QC samples for water, sediments, tissues (biological) and air are discussed.

Chapter 4: Sampling procedures

This chapter provides details on the sampling procedures for water, sediments, tissues (biological) and air, respectively. Details are given on pre-field preparation, site selection, sample collection, transport, storage and analysis.

Chapter 5: Data capture sheets

Examples of field data capture sheets are provided and the advantages of logging all information and recording the chain of custody are described.

Chapter 6: Other considerations

This chapter addresses issues such as logistics, sampler training, the shortage of labs and costs.

Analysis categories

Analysis of EDCs in environmental matrices has been problematic due to both the large numbers of compounds with endocrine disrupting capabilities that may be present in the environment and the ultra-low concentrations. Chemical methods have always been used as screening tools; however, biological methods are becoming increasingly popular. Bioassays are an important component of examining the presence of and integrating the effects of complex mixtures of endocrine disrupting chemicals. For detailed analytical procedures, the following documents should be consulted:

- For bioassays – consult WRC Report No. 1816/1/10 by De Jager C, Aneck-Hahn NH, Barnhoorn IEJ, Bornman MS Pieters R, Van Wyk JH and Van Zijl C, entitled The Compilation of a Toolbox of Bio-Assays for Detection of Estrogenic Activity in Water.
- For inorganics – consult WRC Report No. K8/999 by Meyer JA, Heystek L, Gerber M, Hudson-Lamb D, Havenga S, Matema T and Van Rooyen, entitled Sampling and Methods for the Analysis of Inorganic Endocrine Disrupting Chemicals**.**
- For organics – consult a report written (WRC Report No. 1816/1/10) as part of the WRC Consultancy K8/920 by Burger AEC, entitled Guidelines for Chemical Analysis of Endocrine Disrupter Chemicals in Water Resources.

CHAPTER 2

SAMPLING PROGRAMME DESIGN

A sampling programme is the process of collecting samples and preparing them for analysis. This is a sub-process within an overall broader study programme. The sampling programme should help achieve the objectives of the study programme with the minimum resources (SANS 5667-1:2006). The resources may be personnel, time, and financial.

Objectives of a study programme

Typical objectives of a study programme are to:

- Establish whether or not contamination levels exceed a threshold of unacceptable risk.
- Identify the location of 'hot spots' (areas having high levels of contamination) or plume delineation.
- Characterise the nature and extent of contamination at a site.
- Monitor trends in environmental conditions or indicators of health.
- Describe the baseline conditions in order to differentiate between naturally occurring EDCs and those due to contaminants and to contextualise the relevance of EDC effects compared to toxicological and/or carcinogenic effects.

Designing a sampling programme

Designing a sampling programme is primarily influenced by the overall objectives of the broader study programme. These objectives will establish, for example, what water quality constituents are relevant and what analytical methods are necessary. These in turn determine the sampling and sample preparation that is necessary.

A well-designed sampling programme should answer the following questions:

- How are samples to be collected?
- When are samples to be collected?
- How much sample is to be collected?
- Where the samples must be collected?
- How often should they be collected?

Proper analysis of samples is indispensable when selecting a proper sampling programme. Therefore selection of a laboratory that will undertake sample analysis is very important. Preferably analyses should be performed by a laboratory that has been accredited under the International Standard

ISO/IEC 17025 which is a laboratory accreditation system. Accreditation is seen as the most effective way of giving formal recognition to laboratories that are competent to carry out specific tasks (van Niekerk, 2004).

An accredited laboratory should be able to demonstrate the following basic requirements:

- The laboratory must be able to prove competence for infrastructure, and instrumentation, and must demonstrate that it has well-trained staff to conduct specific analyses.
- Validation of the analytical methods including in-house methods.
- Procedures for the validated methods or standard operating procedures (SOPs), including all the laboratory equipment and consumables.
- Quality criteria for quality assurance and quality control (QA/QC) described in the SOPs, such as analysis of blank samples, use of reference materials, and sensitivity of the analytical system.

Before making a final decision on the laboratory that will undertake sample analysis, it is suggested that the following items be discussed with candidate laboratories:

- **Confirmation of analysis:** Ask the laboratory to confirm that they are able to analyse each of the compounds of interest (name each one), and the range of sample types you plan to collect (i.e., soil, water, air, tissues, etc.). The laboratory should also confirm that they have accreditation for all these sample types and analyses.
- **Limits of detection (LOD):** Ask the laboratory to specify its limits of detection for each of the chemicals in each type of sample, and how these compare with commonly-used environmental standards.
- **Cost:** This will most likely be important in determining the total number of samples to be collected (given limited budgets). Also note that it will be helpful to give the laboratory an initial idea of how many samples you expect to collect, as some laboratories give a discount for larger sample numbers.
- **Sample containers and sampling instructions:** Most samples intended for EDC analysis should be collected in specially cleaned containers to avoid the risks of contamination. The containers should also be leak-proof and relatively robust for shipping purposes.
- **Presentation and interpretation of results:** Ask how the results will be presented and whether any interpretation will be provided, such as checks against recognised standards.

Initiating a new sampling programme

When a new programme is being initiated, it is useful to start with a small-scale pilot project or preliminary survey. This helps to:

- Provide an opportunity for newly-trained staff to gain hands-on experience and to confirm whether or not each component of the programme can be implemented as planned.
- Provide an opportunity to assess the sampling network and provide indications of whether more (or possibly fewer) samples are needed.

- Refine the logistical aspects of the programme such as access to sampling sites, to establish whether refinements are necessary to site selection (Mäkelä and Meybeck, 1996).

CHAPTER 3

QUALITY ASSURANCE (QA) AND QUALITY CONTROL (QC)

The main function of any water monitoring programme is to ultimately produce data or information that will in some way be used to support water management decisions. The social, environmental and financial implications of making incorrect decisions as a result of unreliable data or information can be severe. Unreliable data or information gaps are a direct result of a monitoring programme with a poorly designed or maintained quality assurance programme (Van Niekerk, 2004). A well-designed and consistently implemented QA/QC programme will ensure that the data are credible and of known reliability. See the glossary for definitions of QA and QC.

Effective QA/QC procedures and a clear delineation of responsibilities are essential in ensuring the validity of environmental monitoring data. As pointed out in Dines and Murray-Bligh (2000), the QA/QC procedures must also form an integral part of monitoring activities to ensure the representativeness and integrity of samples and that the resulting data used in review and reporting are accurate and reliable.

QA/QC samples are collected in addition to the regular samples in the programme design. The types of samples to be collected and sampling frequency depend on:

- study objectives
- data-quality requirements
- site conditions
- management or regulatory policy

These samples can also be used to measure data, identify data quality problems and establish their causes.

Types of water quality QA/QC samples

Blank samples generally containing deionised water, are used to determine if contamination might enter a water sample as a result of sampling-related activities. The following are different type of blank samples:

- **Trip blanks -** usually prepared in the laboratory and simply travel with the sample bottles from the laboratory to the sampler, to the sample site, and then back to the laboratory w*ithout ever being opened*. Trip blanks help determine contamination within the bottle or from volatile compounds.

- **Field blank sample -** prepared in the same manner as a trip blank and makes the journey as a trip blank; however, the difference arises when sampling occurs. During the sampling process, the *field blank sample is opened and the collection process is mimicked*. These samples will help determine contamination from bottles, collection methods, the atmosphere, and preservatives.

- **Filtration blanks** - should be used regularly or as a minimum, when contamination is suspected. These measure contamination from the filters, and the filtration apparatus.
- **Bottle blanks** - measure contamination from improper cleaning of bottles. As for filtration blanks, bottle blanks need only be used when contamination is suspected (CCME, 2011).

Types of sediment QA/QC samples

- **Station replicate samples** - these replicate sample collection at sampling sites. Station replicate samples can be collected to determine the variability of the concentrations of contaminants in the sediment at a specific site and/or as an assessment of field sampling techniques.
- **Field duplicate** - duplicates are collected by 'splitting' a sample that has already been collected into two identical samples for analysis. Field duplicate samples are collected to determine laboratory analytical variability and/or field compositing techniques and of sediment heterogeneity within a single collected sample.
- **Equipment rinse samples** – water or solvent used to rinse the sampling equipment between samples are used to determine contamination introduced through contact with sampling equipment.
- **Field blank samples** – field blanks are samples of uncontaminated silica sand collected using the same sampling equipment and techniques as the sediment sample collections. The field blank samples are used to demonstrate that significant amounts of contaminants are not introduced into the sediment samples from sampling equipment or sample handling (CCME, 2011).

Types of biological QA/QC samples

- **Replicate samples -** biological replicate samples can consist of multiple samples (grabs, tows, or whole fish) from the same general area. This is mainly to measure how well a single sample represents the community or how many samples are necessary to achieve some level of sampling confidence, or portions of a single sample (i.e. sectioned grabs - to measure more localized invertebrate heterogeneity).
- **Split samples** – are aliquots taken from the same container and assumed to be identical. These samples can be sent to two or more laboratories for separate analysis and the results can be used to determine inter-laboratory variability or the consistency of results within one laboratory.
- **Reference samples** - laboratory-tested and preserved reference materials are available for tissue samples. These reference tissues have been subjected to a large number of analyses performed by independent laboratories using several different analytical techniques.

- **Taxonomy samples** – reference samples should be submitted to the analysing laboratory along with the samples collected during a field trip. They should be transferred to a regular sample container and labelled with plausible site names and numbers (the codes used for identification must be documented in the field logbook). The US EPA is a source of these taxonomic reference materials, at least for algal taxonomy, chlorophyll *a* and several bacterial species (CCME, 2100).

Types of air QA/QC samples

- **Blank samples** - to determine if the sample tubes were contaminated during manufacture, packaging and/or shipping.
- **Duplicate samples -** duplicate samples are used to check and verify the accuracy, precision and consistency of the laboratory analyses.
- **Run blanks** - should be undertaken per monitoring campaign to check that the installation methodology is not contaminating the sample. To undertake the 'run blank' the sampler is installed into the monitoring equipment and then immediately removed and sent to the laboratory for analysis.

CHAPTER 4

GENERAL CONSIDERATIONS

General pre-field preparation

- Ensure that any necessary permits and/or permissions are obtained.
- Ensure that all necessary equipment is available and in good working order.
- Ensure that all containers are properly cleaned.

General site selection criteria

Sample site selection is best done prior to sampling. However, this is not always practically feasible. In some instances sample site selection will follow a process of elimination after evaluation of the analytical results. Sample sites can also be selected by determining the location of so-called 'hotspot' areas. The following general guidelines apply:

- Sites where industrial pollution is known to have been detected.
- Sites where considerable agricultural activity takes place.
- Sites where activities such as malaria control take place.
- Sites where effects on humans and wildlife have been noted.
- Sites where research is already being done and from which additional data may therefore be available (SANS 5667-1, 2006).

Additional details regarding considerations applicable to the sampling protocol in terms of site and sample site selection are provided in Volume IV: Monitoring and Assessment of this EDC guideline volume series**.**

General sample collection procedures

As each laboratory may have specific sample collection procedures it is advisable to first check with the laboratory regarding the following aspects:

- The preferred collection methods.
- The types of sample containers provided and prepared by the laboratory.
- Sample delivery.
- Sample-delivery schedule.
- How to label the sample containers.

This will assist in testing the operational approaches as well as help to establish the means of transport, on-site testing techniques and sample preservation and transport methods. It will also assist the lab in planning properly.

Laboratory selection criteria

It is important that the selected laboratory is capable of performing the analysis using internationally acceptable analytical methods, and has well documented QA/QC systems in place. Several methods have been described for the analysis of these compounds. Consult Burger (2005) for details on laboratory selection.

Safety issues

- Proper protective clothing should be used all the times.
- Physical hazards must be identified.
- Samplers should be familiar with safety policies.

CHAPTER 5

SAMPLING PROCEDURES

WATER

This section details the methods for correct sample collection, storage and transportation to allow for appropriate analytical work to be performed for organic and inorganic EDCs in water.

Pre-field preparation

New sample containers are strongly recommended for EDC analytical procedures. It is, however, recognised that containers may be appropriately cleaned for reuse in the case of macro-element determination, especially for routine monitoring. More details regarding cleaning of sample bottles for inorganic EDCs are provided in the report by Meyer JA, et al (2012).

- Generally sample bottles used for EDC sampling should be washed with soap and water, and then rinsed with methanol.
- Amber glassware with Teflon-lined screw cap should be used as it has the least impact on the target analytes compared to high density polyethylene (HDEP) bottles where many of the target compounds show poor recoveries.
- Plastic containers must be used for inorganic EDCs, i.e. macro- and trace elements, and glass containers for mercury (glass is generally not recommended due to potential leaching of boron and fluoride and breakages, but is, however, required for mercury analysis).
- Coordinate the sample collection process with the laboratory and refine the sample volume requirements and preservation methods accordingly.

Site selection

Sampling can be complex in locations where wide, rapid and continuous variations occur in characteristics such as the concentrations of constituents of interest. These variations can be caused by factors such as extreme changes in temperature, flow patterns, and plant operating conditions. Sampling sites are usually selected by personal judgment, knowledge of the geography of the area and of any discharges of wastes (SANS 5667-1, 2006).

When selecting a sampling site, the following factors should be considered:

- Accessibility to the selected site.

- Safety measures usually associated with the operation of the sampling equipment.
- Hazards such as glass or sharp objects lying out of sight under the water.
- Ability to accurately locate the sampling site.

Sample collection

The most common and simplest method used for water sampling is grab sampling. For this method, samples are taken by holding the collection bottle and lowering it into the water until covered. Sample volume depends on the compounds being analysed. Generally a 1 ℓ sample from each site will suffice. Figure 1 illustrates the correct way of taking a grab sample.

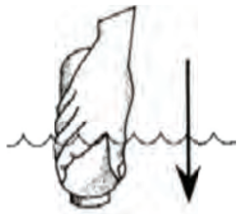
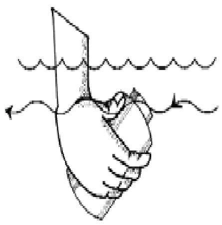
Step 1  At the sampling point remove cap of sample bottle but do not contaminate inner surface of cap and neck of sample bottle with hands.	Step 2  Take samples by holding bottle with hand near base and plunge the sample bottle, neck downward, below the water surface (wear gloves to protect your hands from contact with the water).
Step 3  Turn bottle until neck points slightly upward and mouth is directed toward the current (can also be created artificially by pushing bottle forward horizontally in a direction away from the hand).	Step 4  Fill sample bottle without rinsing and replace cap immediately.

Figure 1: Sample collection techniques (Quality of Domestic Water Supplies – Volume 2: Sampling Guide. WRC Report No. TT117/99*)*

When sampling from water bodies, the following safety issues must be considered:

- Adequate precautions must be taken to ensure the safety of the sampling team.

- Be aware of possible bank failure that may cause loss of balance.
- The person sampling must wear a lifeline. All sampling personnel must wear personal flotation devices (life vests).

Sample storage and transport

- Samples must be cooled immediately after sampling and kept below 4°C.
- The vehicle used for transportation should preferably be fitted with a refrigerator; alternatively cooler boxes may be used.
- It is preferable that samples reach the laboratory within 48 h (SANS 5667-2, 1991).

Table 3 indicates the sample volume, type of bottle to be used, sample treatment and sample holding time to be considered when undertaking sampling for EDCs in water.

Table 3: Samples bottles, preservative and holding times for sampling EDCs in water

Compounds	Sample volume	Bottle	Preservative	Treatment	Max. holding time
Organochlorines Organophosphates Pyrethroids Carbamates Herbicides Fungicides PCBs Alkylphenols Synthetic hormones Natural hormones Hormones Plasticisers Dioxins Dibenzofurans	1 ℓ	Amber glass bottle with Teflon-lined cap	No preservation	Store below 4°C in the dark	Samples must be extracted within 7 days of sampling and extracts must be analysed within 40 days from day of sampling.

SEDIMENT

Sediments are regarded as inorganic and organic material that is suspended in and transported by surface water or that has settled out and been deposited. Contaminated sediments can degrade ecological integrity and pose a threat to human health when pollutants bio-accumulate in edible aquatic organisms. When undertaking sediment sampling, it is important that control points should be identified so as to act as a reference point in determining the levels of contamination against background levels (DEP, 2001).

The following aspects should be considered when sampling sediments:

Depth of sampling

- Site history and possible depth of contamination through deposition.
- Nature of contaminants (mobility, persistence).
- Known or assumed maximum depth of contamination.
- Field observations and identification of contamination (e.g., stained sediments).
- Diffuse or point-source contamination sources (diffuse contamination within a harbour, or point-source contamination at depth from a pipe discharge)
- Potential for mixing down the sediment profile.
- Human health and ecological risks.

Number of samples

The number of samples to be collected should also take the following aspects into consideration:

- Objectives of the project.
- Size of the area to be sampled.
- Sampling pattern applied.
- Saturation, complexity and distribution of known contaminants.
- Small-scale variability in contaminant concentration.

Frequency of sampling

There is often some form of mobility of sediments, and therefore more than one sampling event may be required to build up a picture of temporal changes in sediment quality.

Pre-field preparation

- Stainless steel or enamel-coated containers should be used to collect sediment samples.
- Amber glass jars with Teflon-lined screw caps should be used for sample storage.
- A boat might be used, depending on the distance away from the banks.

Site selection

Sediment sampling is usually an expensive exercise. The following points should therefore inform site selection:

- Availability of information in terms of physical characteristics.
- Potential contaminants.
- Potential sources of contamination.

This information will enable a decision to be made about the potential remobilisation of contaminants into the water column, thus determining the sampling apparatus and techniques to be followed (SOEPA, 2001).

Sample collection

Sediment samples for EDC analysis are usually collected within the top 10 cm of the sediment surface with equipment that causes the least disturbance to the sediment surface during collection. In this case it is suggested that the grab-sampling method be used since it does not disturb the surface sediment. Sediment samples may be collected as follows:

- Collect a minimum of three separate samples within a radius of 5 m to 10 m in the same pre-cleaned stainless steel or enamel-coated container.
- Mix the sample thoroughly by stirring vigorously for 30 s to 60 s with a pre-cleaned stainless steel stirrer to create a representative pooled sample.
- Pour sub-samples into a minimum of three pre-cleaned amber glass bottles. Glass bottles should be covered with Teflon-lined lids or pre-cleaned aluminium foil since the linings of the lids might contaminate the samples. Take care not to overfill glass bottles as they expand when frozen and often crack.
- Collect a minimum volume of 100 g of sediment.
- Label properly with the site name as it appears on the field data sheet (Appendix 1) and laboratory submission form, the date and time of the sample collection and the name of the sample collector or other information that may have been specified by the laboratory *(www.popstoolkit.com/sops/methods/sediment)*.

Step 1

Always wear new, clean nitrile gloves while sampling.

Step 2

Thoroughly clean all sampling equipment (mixing tray, spoon, etc.) with metals-free soap and deionised water prior to sampling at each site. Where analyses require, use chemical solvents such as hexane and acetone to ensure all residues are dissolved from equipment surface.

Step 3

The sampler should wade into the pond until the desired depth is reached, and then scoop sediments directly into sampling jars, or by using a stainless steel pan.

Step 4

Collect a minimum of three separate samples within a radius of 5 m to 10 m in the same pre-cleaned stainless steel or enamel-coated container and using the stainless steel spoon, mix the sediment sample until it is thoroughly combined into a single homogeneous sample in a mixing tray.

Step 5

Pour sub-samples into a minimum of three pre-cleaned glass bottles with Teflon lid. Take care not to overfill glass bottles as they expand when frozen and often crack.

Step 6

Label properly. Ensure that the label on the container includes the sampling name, date of sampling, and type of sample.

Figure 2: Procedure for sediment sampling (www.popstoolkit.com/sops/methods/sediment)

When sampling sediments, special attention should be given to the following:

- Be careful not to slip, trip or fall.
- Avoid skin contact with sediments.
- Inhalation of odours should be avoided.
- Special precautions may have to be taken when working with contaminated sediments especially near potential or known contaminant sources.

Transport and storage

- Samples should be transported to the laboratory on ice at a temperature of below 4°C.
- The samples should be kept cool and dark in the laboratory until the analysis is completed (Spectrum Analytical Inc, 2010).

See Table 3 above for information on sample volume, type of bottle to be used, sample treatment and sample holding time to be considered when undertaking sampling for EDCs in sediments. This information is similar as when handling water samples for EDC analysis.

BIOLOGICAL (FISH TISSUE)

Fish serves as an important food source to humans and other animals. Most communities that have access to freshwater practise recreational fishing. EDCs can accumulate in fish which can then pose health risks to humans and animals eating the fish (Heath et al., 2004). It is, however, recommended that a suite of tissue types be used because some inorganic EDCs may result in endocrine disruption not only by direct effects, but by induced deficiencies as well.

Pre-field preparation

- Appropriate sample containers for biological samples are glass jars or glass vials with screw caps. Screw caps should be lined with solvent-rinsed aluminium foil or Teflon cap inserts.
- Clear polyethylene bags, and polypropylene jars are also appropriate for packaging and temporary storage of whole fish, but may not be suitable for long-term storage because of the possibility of migration of plasticisers (such as phthalates) into the tissues, especially for samples with high lipid contents.
- Blood samples from the fish should be collected in ethylenediamine tetraacetic acid (EDTA) vials or Vacutainers.

Site selection

The following should be considered:

- Sampling site should be located near point sources of pollution (e.g., municipal, industrial or mine discharges) or diffuse sources of pollution.
- Select potentially unpolluted areas to be used as reference points; these sites should be located either upstream or downstream of the sampling point.

Other considerations should include:

- The type of sampling equipment.
- Accessibility to the site.
- Time/season of sampling (some fishes are known to migrate in different seasons).
- Barriers to migration (dams and waterfalls).
- Permits to collect fish.

Sample collection

Different fishing methods are currently available to collect fish from freshwater. The method employed will depend on:

- The specific water body.
- Human resources available.
- The type of equipment available.

It is extremely important to avoid subjecting fish to unnecessary stress such as inappropriate capture and transport, temperature shock or water quality change. Depending on the sampling circumstances, fish can either be processed in the field or transported to the laboratory. It is highly recommended that fish be processed in the laboratory so as to avoid contamination of samples. A number of fish collection methods are well documented and available in South Africa (Table 4) (Heath et al., 2004).

Table 4: Fish collection methods that can be used in South Africa (Heath et al., 2004)

Equipment	Area of use	Advantages
Gill nets	Lakes, rivers and reservoirs	• Effective for pelagic fish. • Easy to operate and fishing effect reduced. • Selective catches due to use of different mesh size.
Seine nets	Lakes and shallow rivers	• Relatively inexpensive and easy to operate. • Selective catches due to use of different mesh size. • Unwanted fish can be returned unharmed.
Electro-fishing	Lakes and shallow rivers	• Efficient non-selective method. • Minimal damage to fish. • Adaptable to a number of conditions, e.g. wading and from a boat.
Hook and line	Lakes and shallow rivers	• Most selective method. • Equipment not too expensive. • Large number of personnel not required.
Purchasing species from fishermen	Only in cases where selected species are harvested commercially or by subsistence fishing	• Most cost-effective and efficient method to obtain commercially valuable species.

After fish collection:

- Record the weight, length and species of the fish.
- Wrap the whole fish in aluminium foil and place in polyethylene bag.

The following health and safety tips should be adhered to:

- Take appropriate precautions when operating a boat and working in and around water.
- Use caution when handling fish as the fins, gills and teeth of some species are sharp.
- Tissue sample processors must use caution when using scalpels.

Fish tissue sampling

Before tissue harvesting begins:

- The scales should be removed first by scraping with the edge of a knife and then rinsing the fish to remove slimes.
- If a scale-less fish is used in the study, then the skin should be removed by grasping the head of the fish and removing the skin with pliers while pulling from the head toward the tail *(www.popstoolkit.com/sops/methods/fish).*

Figure 3 illustrates a step-by-step procedure on how to remove tissues from fish.

Step 1

Measure the length (mm) and weight (g) of each fish.

Step 2

Remove the skin using a pair of tweezers and the scalpel. Do not touch the dissected area with gloves.

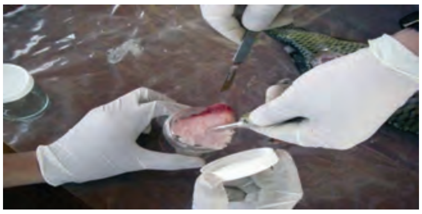
Step 3

Make incisions around the dissected area to free the muscle from the fish.

Step 4

Carefully remove the muscle tissue sample from the fish, making sure no skin or scales are included in the sample.

Step 5



Transfer the sample to a clean laboratory-supplied jar, taking care not to touch the sample with your hands or any foreign object.

Step 6

Write the sample name and type of sample on the cap of the jar.

Figure 3: Procedure for fish tissue sampling (www.popstoolkit.com/sops/methods/fish)

Fish blood sampling

Blood may be sampled using four different procedures:

- Dorsal aorta.
- Cardiac puncture.
- Caudal vein.
- Caudal severance.

Of the four sampling methods the caudal vein is the preferred method for fish. A 22-gauge hypodermic needle is recommended for larger fish. Smaller gauge needles (upwards of 22 gauge) are recommended for smaller fish.

Caudal vein:

The sample is taken midline just posterior of the anal fin. Insert the needle into the musculature perpendicular to the ventral surface of the fish until the spine is reached or blood enters the syringe. If contact with the spine is made, withdraw the needle slightly. The vein is ventral to the overlying spine. This blood vessel can also be

Dorsal aorta:

Insert needle at a 30° to 40° angle into the dorsal midline in the roof of the mouth at about the 3rd to 4th gill arch. Depending upon size and species of fish insertion between the 1st and 2nd arch may be more suitable.

Cardiac puncture:

Blood is collected from the heart ventricle. Insert needle perpendicular to the ventral surface of the fish in the centre of an imaginary line between the anterior-most part of the base of the pectoral fins.

Caudal severance:

Dry the caudal peduncle. Completely sever the tail posterior to the anal fin. The first few drops are discarded; the rest is collected in microhematocrit tubules.

Figure 4: Procedures for fish blood sampling (Ferguson, 2005)

Transport and storage

- Individually wrapped fishes must be transported to the laboratory whilst kept at 4°C.
- Whole fish can be kept frozen at -20°C in the dark prior to tissue sampling.
- Tissues can also be stored at the same temperature until analysis.

Table 5 provides information on sample volume, type of bottle to be used, sample preservation, treatment and sample holding time to be considered when sampling fish blood and tissues for EDCs analysis.

Table 5: Samples bottles, preservative and holding times for sampling EDCs from fish tissues and blood

Compounds	Sample type	Bottle	Preservative	Treatment	Max. holding time
Organochlorines Organophosphates Pyrethroids Carbamates Herbicides Fungicides PCBs Alkylphenols Synthetic hormones Natural hormones Hormones Plasticisers Dioxins Dibenzofurans	Fillets and homogenates Blood	A wide-mouth glass jar with Teflon-lined screw top Ethylenediamine tetraacetic acid (EDTA) vials or Vacutainers	Freeze at ≤ 20°C	No treatment	28 days after sampling/ extraction before analysis

AIR

Air is an important matrix because it has a very short response time to changes in atmospheric emission and is a relatively well-mixed environmental medium. Many compounds in air can transfer to humans and wildlife and have been linked to adverse health effects, even at low concentrations. The concentrations and mixtures of EDCs in the atmosphere vary widely in space, time and with compound class. Due to low concentrations in the atmosphere, a large volume of air needs to be sampled to facilitate detection of these compounds (Ockenden WA *et al,* 2001). Two techniques for air sampling are available:

- **Active sampling** involves the use of an air-sampling pump (mostly electrically powered) to actively pull air through a collection device such as a filter. The disadvantages of active sampling techniques are that expensive equipment is required and that trained operators are required on hand to run the equipment.
- **Passive sampling** does not require electrical power. This technique samples the atmospheric species by means of gaseous diffusion and sorption and/or permeation. The main advantage is that passive samplers are generally cheaper and easier to use than active sampling devices and they have been used successfully over a range of geographical scales.

Pre-field preparation

- Prior to use, the polyurethane foam (PUF) disks and styrene/divinylbenzene-co-polymer (XAD-2) resin must be pre-cleaned by sequential Soxhlet extraction using acetone, hexane and/or acetone followed by hexane and then dried under vacuum.
- Amber glass jars with Teflon-lined screw caps are required to store the samplers prior to and after deployment.
- The air samplers must be calibrated before deployment to allow for a precise measured volume of air to pass through the sampling media. The volume of air will depend on the length of deployment and the properties of the compound (Jaward FM, 2004).

Site selection

When selecting sites for EDC sampling, consider the following:

- Processes responsible for the observed air concentrations
- Trends at the site.
- The regional and global-scale transport of air particles; to do this, an understanding of the local (meso-scale) as well as large-scale (synoptic) air-transport pathways to the selected site is required.

Again, the availability of laboratories capable of atmospheric measurements and consideration of sources and air-transport pathways will influence the site selection. The following general criteria should be considered when selecting sites for air sampling depending on the objectives of the programme:

- **Regional representivity:** A location free of local pollution sources, such that air sampled is representative of a much larger region around the site.
- **Minimal meso-scale meteorological circulation influences:** Free of strong systematic diurnal variations in local circulation imposed by topography (e.g., upslope/downslope mountain winds and land/sea/lake breeze circulation).
- **Long term stability:** This includes infrastructure, institutional commitment, and land development in the surrounding area.
- **Appropriate infrastructure and utilities:** Accessibility, buildings, platforms, towers and roads.

Sample collection

- Insert the disks in the steel chambers (Figure 5).
- Allow air to flow over the sampling surface through a gap between the two domes and through holes in the bottom surface of the lower dome.
- Insert one field blank in the sampling chamber and immediately remove and store in an amber glass jar with lid.

Disks can be deployed at sampling sites for 60 to 160 days.

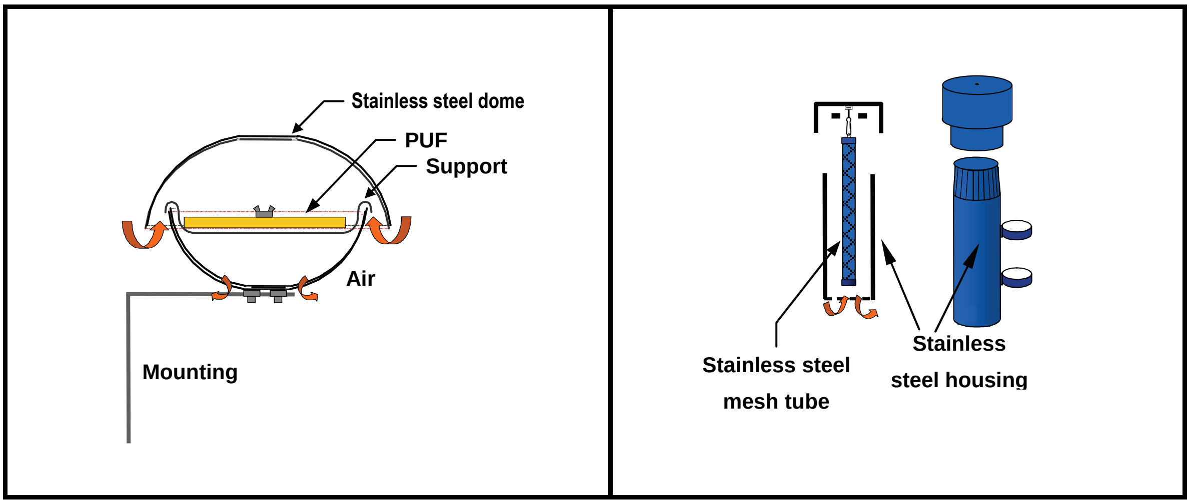


Figure 5: A representation of the PUF Disk (left) and XAD type (right) air-sampling devices (Jaward et al., 2004)

Transport and storage

After sample collection:

- Store disks in solvent-rinsed 1 ℓ amber glass jars with Teflon-lined screw caps.
- Seal and store below 4°C.
- Transport to the laboratory for extraction and analysis.

Table 6 provides information on the suggested type of filter to be used when sampling EDCs in air. In each case the samples should be stored at 4°C in the dark. No treatment is required. The samples must be extracted within 7 days and extracts must be analysed within 40 days of sampling.

Table 6: Filter types for sampling EDCs in air

Compound	Filter types
Organochlorine pesticides	Low volume air sampler, PUF and/or PUF/XAD-2 adsorbent cartridges
Carbamates	Sorbent tube or PUF (22 X 100 mm)
Pyrethroids	NIOSH Tenax tube (not a reference method but can be used)
Herbicides	Filter/solid sorbent tube (OVS-2 tube: 13 mm quartz fibre filter; XAD-2, 270 mg/140 mg)
Fungicides	Filter/Solid Sorbent Tube (OVS-2 Tube: 13 mm quartz fibre filter; XAD-2, 270 mg/140 mg)
PCBs	Low volume air sampler, PUF and/or PUF/XAD-2 adsorbent cartridges
Alkylphenols	Low volume air sampler, PUF and/or PUF/XAD-2 adsorbent cartridges
Plasticisers	FILTER (0.8 µm cellulose ester membrane)
Dioxins and Dibenzofurans	XAD-2 and PUF cartridges
Benzo(a)perene	Filter plus sorbent tube (2 µm, 37 mm PTFE plus washed XAD-2, 100 mg/50 mg)
Styrene	Filter/solid sorbent tube

CHAPTER 6

DATA CAPTURE SHEETS

Field data sheet

Good sampling practice requires making detailed field notes. Specific information about seemingly unimportant facts, such as the time of day or weather conditions, can be significant when interpreting data. In addition to documenting standard conditions and measurements, field personnel are responsible for noting any unusual occurrences. Any deviations from standard protocols such as samples taken from a different location due to safety issues or other considerations, such as access constraints, should also be recorded. See Appendix 1 for examples of field data sheets.

Advantages of keeping field notes

- Field notes can be critical in the interpretation of the data.
- Notes can be reviewed prior to completing additional sampling.
- Previous notes provide insight into the preparation of a sampling trip and the possible hazards or conditions that a site may present.

Details recorded

The details recorded on the field data sheet will vary according to the objectives of the sampling programme. However, details should include all the information necessary to assist in data interpretation and repeatability of the sampling exercise. Information that could be considered for inclusion on the field data sheets includes but is not limited to the following:

- Name and location of the sampling point.
- Date and time of sample collection.
- Any relevant descriptive information such as water level or flow.
- Sample appearance at the time of sampling such as colour and odour.
- Field parameters measured.

Chain of custody

This is a process that details the link in the transfer of samples between the time of collection and arrival at the designated laboratory. Depending on the design of the sampling programme several transfers may take place in this process, for instance, from the sampler to the courier and from the courier to the laboratory (OECD, 1998). The following basic information should be included on the chain-of-custody form:

- Name of person transferring samples.
- Name of person receiving the samples.
- Time and date the samples were taken.
- Time and date the samples were received in the laboratory.
- Condition of the samples, e.g., chilled.
- Name and contract details.
- Analytes to be determined.
- Details of the sampling matrix.

CHAPTER 7

OTHER CONSIDERATIONS

Logistics

Given the limited number of laboratories that are capable of analysing for EDCs, it is important to consider the location of the laboratory in relation to sample collection. Long distances can affect the type of container used, storage and handling of the samples.

Sampler training

Toxicity testing is relatively complex and demanding. Sample collection is a critical component. Samplers should be well trained on how to properly collect samples that are intended for EDC analysis.

Lack of laboratories

A number of surveys on analytical capability have been conducted. However, in 2004, Burger and Heath conducted a survey which revealed a somewhat diminished capacity compared to previous surveys. For this reason, a survey to establish laboratory capacity was again performed during this study and an updated list of laboratories capable of analysing these compounds is included in Appendix 2. A table for laboratories that are capable of analysing for EDCs to provide their details is attached as Appendix 3.

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www.popstoolkit.com/sops/methods/sediment - Techniques for sediment sampling.

APPENDIX 1

Examples of field data sheets

APPENDIX 1.1:

WATER FIELD-DATA SHEET

STUDY PROGRAMME:	
Site name:	Date:
Sample collected by:	Field notes recorded by:
Start time:	
Finish time:	
Water collection method:	
Sampling device used:	
Colour:	
Texture (e.g., rocky, sandy) :	
Storage conditions:	
Analysis (e.g., organics, inorganics or bioassays):	
Description of the sampling point:	
Parameters to be tested for: (standard list to be provided where parameters required will be ticked off)	

APPENDIX 1.2:

SEDIMENT FIELD-DATA SHEET

STUDY PROGRAMME:	
Site name:	Date:
Sample collected by:	Field notes recorded by:
Start time:	
Finish time:	
Sediment collection method:	
Sampling device used:	
Colour:	
Texture (e.g., rocky, sandy) :	
Storage conditions:	
Analysis (e.g., organics, inorganics or bioassays):	
Description of the sampling point:	
Parameters to be tested for: (standard list to be provided where parameters required will be ticked off)	

APPENDIX 1.3:

FISH FIELD-DATA SHEET

STUDY PROGRAMME:	
Site name:	Date:
Sample collected by:	Field notes recorded by:
Start time:	
Finish time:	
Fish-capture method:	
Fish species:	
Weight:	
Length:	
Sex:	
Tissue collected: Muscle Fat Liver	Abnormalities noted:
Storage conditions:	
Analysis (e.g., organics, inorganics or bioassays):	
Description of the sample point:	
Parameters to be tested for: (a standard list to be provided where parameters required will be ticked off)	

APPENDIX 1.4:

AIR FIELD-DATA SHEET

<table>
<tr><td colspan="2">STUDY PROGRAMME:</td></tr>
<tr><td>Site name:</td><td>Date:</td></tr>
<tr><td>Sample collected by:</td><td>Field notes recorded by:</td></tr>
<tr><td>Start time:</td><td></td></tr>
<tr><td>Finish time:</td><td></td></tr>
<tr><td colspan="2">Air collection method:</td></tr>
<tr><td colspan="2">Sampling device used:</td></tr>
<tr><td colspan="2">Date manufactured:</td></tr>
<tr><td colspan="2">Storage conditions:</td></tr>
<tr><td colspan="2">Analysis (e.g., organics, inorganics):</td></tr>
<tr><td colspan="2">Description of the sampling point:</td></tr>
<tr><td colspan="2">Parameters to be tested for:

(list of parameters to be provided where analysis required will be ticked off)</td></tr>
</table>

APPENDIX 2

Analytical laboratories performing EDC analyses

	Analysis			Matrix				
Laboratory/organisation	Organics	Inorganics	Bioassays	Water	Sediment	Biological	Air	Website
Air Separation	X			X	X	X	X	http://www.airseplabs.co.za
AMPATH	X	X	X			X		http://www.ampath.co.za
ARC (Plant protection Research Institute)	X	X		X	X	X		http://www.arc.agric.za
Aquavan laboratory	X	X			X			
CSIR (Biochem/Tek)	X			X	X	X		http://www.csir.co.za/environmental
		X		X	X			
CSIR (Environmentek)	X	X	X	X	X	X		http://www.csir.co.za/environmental
CSIR NRE Environmental Analytical Laboratories	X	X		X	X	X		http://www.csir.co.za/nre/
ERWAT	X	X		X				http://www.erwat.com
Durban Institute of Technology	X			X	X			http://www.dut.ac.za/
		X		X				
Inspectorate South Africa	X	X		X	X		X	http://www.inspml.co.za/
North West University			X	X	X	X	X	http://www.nwu.ac.za/content/zoology-popt-index
Protechnik Laboratories	X	X	X	X	X	X	X	http://www.armscordi.com
Rand Water	X	X		X				http://www.randwater.co.za
South African Bureau of Standards (SABS)	X	X	X	X	X	X	X	https://www.sabs.co.za/
Umgeni Water	X	X		X				
UIS Organic Laboratory	X			X	X			http://www.uisol.co.za/
University of Cape Town	X			X	X	X	X	http://www.uct.ac.za/
			X			X		
University of Pretoria			X	X		X		http://web.up.ac.za/

APPENDIX 3

Laboratory information sheet

Laboratory/organisation	Analysis	Matrix	Contact person	Contact number