National Toxicity Monitoring Programme (NTMP) for Surface Waters: Phase 2

Prototype Implementation Manual

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EXECUTIVE SUMMARY

Scope and objectives

This is a **prototype implementation manual** for the National Toxicity Monitoring Programme (NTMP) for surface waters. It describes definitively how the NTMP should be implemented in South Africa from a broad national perspective to a detailed procedural level. It is a "prototype" manual because it remains to be subjected to testing in a pilot phase. Although the Department of Water Affairs and Forestry (DWAF or "the Department") has the primary responsibility, this manual is aimed at all who will be involved, both inside and outside the Department. The associated Record of Decision Report [DWAF, 2005a] should be consulted for more detail on the rationale behind many of the design decisions and procedures.

The NTMP monitors water quality status and trends on a national basis in inland surface waters. The design will be extended in future to groundwater and estuaries. The perspective is deliberately strategic and of relatively low resolution. The NTMP is primarily focused on protection of domestic water users and on protecting aquatic ecosystem integrity. The following are the specific objectives.

National Toxicity Monitoring Programme
DWAF National Objectives

To measure, assess and report on a regular basis
on the status and trends of the nature and extent of,

first, the potential for toxic effects to selected organisms, and,
secondly, selected potentially toxic substances
in South African inland surface water resources

in a manner that will
(A) support strategic management decisions
in the context of (1) fitness for use of those water resources
and (2) aquatic ecosystem integrity, and
(B) be mindful of financial and capacity constraints, yet,
be soundly scientific.

Target users

The following are the target users of information from the NTMP:

Primary users:

- The Minister of Water Affairs and Forestry
- DWAF Director General
- Water Resource Quality Managers and Water Quality Managers (DWAF head office and regional offices)
- Water Management Institutions (like catchment management agencies)
- Water User Associations

Secondary users:

- National, provincial and local government authorities
- Non Government Organisations
- All industrial sectors
- Public
- Any other interested party

National coordination

National coordination of the NTMP is the responsibility of the programme manager who will need to be familiar with all aspects of toxicity monitoring. The "hands-on" programme manager should be the driving force behind initial and ongoing implementation on a national basis. He or she will need to anticipate potential problems by consulting managers of other national programmes, examining what problems they encountered and how they were solved. In order to encourage people and organisations to support the NTMP and become involved, early successes should be ensured. Awareness should be created and sustained on a broad basis using appropriate nationwide communication mechanisms.

Participating organisations will need to be managed with care and roles and responsibilities should be well defined. Special attention will need to be given to mechanisms that sustain commitment.

Phased implementation

A phased implementation process is proposed in which new areas are systematically added in which there is a real need. Appropriate catchments can be identified using a prioritisation process. If a catchment management agency is involved, the intended monitoring should be formally incorporated into the catchment management strategy. Sustainability should be facilitated by effective national coordination, ensuring continuity in the regions, regular reporting and periodically reviewing the programme. Reviewing should include the extent to which objectives are being achieved, whether or not the objectives are still relevant, and the appropriateness of all the technical aspects of the programme.

Alignment with classification system

In future, the management class of a water resource will be the overarching concept driving all water resource management and intimately linked to the catchment vision. The NTMP is aligned with this concept to ensure that the information it ultimately provides suitably supplements the "performance" monitoring that will take place to monitor compliance with formal resource quality objectives.

Although the classification system has not yet been developed, the NTMP assumes three ecological categories and water use categories. These have been numbered 1, 2, and 3 to correspond with the three management classes proposed in the National Water Resource Strategy (namely 1 = natural, 2 = moderately used/impacted and heavily used/impacted, 3 = unacceptably degraded). Category 2 deliberately simplifies the situation for purposes of the NTMP by combining the moderately and heavily used classes into a single equivalent category.

Until resources are classified, it is proposed that the ecological category be assumed to be category 1 and the water use category 1 (mainly to be consistent with the approach of other national water quality programmes).

Null hypotheses

Three null hypotheses are used to provide a sound and simple basis for the NTMP design, all implicitly referring to toxicity:

- The resource is not in an acceptable ecological category.
- The resource is not in an acceptable water use category.
- The current status is worse than last year.

These hypotheses allow very simple criteria (relating to both toxicity and toxicant concentrations) to be used to determine whether or not they are true. For the toxicants these take the form of quantitative guidelines. For toxicity these take the form of the existence, or not, of toxicity.

Monitoring variables

In respect of toxicity, the monitoring variables are given in the following tables. For the pilot phase the guppy test will also be used (while the zebra fish test is being phased in) as will the *Vibrio fisheri* test.

Toxicity

Table 1. Toxicity monitoring variables relating to fish (using the semi-static zebra fish development test).

	**Lethality**	**Sub-lethality**
Short-term	% Zebra fish (*Brachydanio rerio*) embryo lethality (96 hours)	
Long-term	% Zebra fish (*Brachydanio rerio*) larval lethality (10 days)	% Effect on Zebra fish (*Brachydanio rerio*) hatching time

Table 2. Toxicity monitoring variables relating to invertebrates (using the *Daphnia pulex* reproduction test).

	**Lethality**	**Sub-lethality**
Short-term	% *Daphnia pulex* lethality (96 hours)	
Long-term	% *Daphnia pulex* lethality (21 days)	% Reproduction inhibition

Table 3. Toxicity monitoring variables relating to algae (using the algal 24-well microplate growth inhibition test).

	**Lethality**	**Sub-lethality**
Short-term		
Long-term		% Alga *Selenastrum capricornutum* Printz growth inhibition

Table 4. Toxicity monitoring variables relating to humans (using the recombinant yeast (hER) test).

	**Lethality**	**Sub-lethality**
Short-term		
Long-term		Recombinant (hER) yeast screen

Toxicants

In respect of toxicant concentrations, the following have been selected for both the pilot phase and the subsequent implementation phase:

Selected POPs relating to the Stockholm Convention

- Aldrin (CAS No. 309-00-2)
- Chlordane (CAS No. 57-74-9)
- DDT (CAS No. 50-29-3) and selected breakdown products (reported as total DDT):
  - DDD
  - DDE
- Dieldrin (CAS No. 60-57-1)
- Endrin (CAS No. 72-20-8)
- Heptachlor (CAS No. 76-44-8)
- Hexachlorobenzene (CAS No. 118-74-1)
- Mirex (CAS No. 2385-85-5)
- PCBs (the Arochlors 1016, 1221, 1232, 1242, 1254, 1260, 1262 and 1268 as commonly reported)
- Toxaphene (CAS No. 8001-35-2)

Other organic toxicants

- Endosulfan isomers (α- and β-) and the sulfate breakdown products:
  - α-endosulfan
  - β-endosulfan
  - Endosulfan sulfate
- Lindane (γ-BHC) and the following isomers:
  - α-BHC
  - β-BHC
  - δ-BHC
- Monocrotophos

- Three triazines:
    - Atrazine
    - Simazine
    - Terbutylazine

New toxicants should only be added if they contribute significantly to achieving the NTMP objectives. Specific criteria have been proposed [Section 5.6, DWAF, 2005a].

Monitoring points

Selection of monitoring points is based on the "priority area" approach while also being consistent with the classification system (when this begins to be implemented). The main factors that establish a priority area are:

- Present land use likely to cause toxicity or result in toxicants in water resources, and
- Sensitive water users are at risk, or
- Important aquatic ecosystems are at risk.

A prioritisation process is proposed for identifying potential priority catchments. This is based on quantifying the degree of toxicity (T), the degree of domestic use (U) and the degree of importance of ecosystems (E). The priority (P) is then calculated from P = T(U+E). Quantification of T, U and E can either be subjectively done (through discussions with regional DWAF officers) or more objectively done (*e.g.* using GIS). Since high accuracy is not necessary, the subjective approach will be adequate. However, resources permitting, the more objective approach can be followed in parallel.

Monitoring points should be at catchment outflows as well as upstream locations if the outflow is not considered to be sufficiently representative of the catchment (*e.g.* because likely toxicants in that region may not be behaving conservatively). The latter monitoring points should be upstream of domestic users or important ecosystems but downstream of potentially polluting land uses.

Monitoring frequency

Because there is a significant lack of monitoring data relating to toxicity, it is not possible at this time to determine the optimum number of samples per year. It is therefore proposed in the interim that monitoring is done as frequently as available resources allow until sufficient data are collected.

Sampling

Rivers should be grab-sampled as far away from the bank as can conveniently be reached from the bank. Stationary water should be avoided. However, in dry periods, large river pools can be sampled. Impoundments or lakes should be grab-sampled away from the shore towards the middle of the water body. A total of eight ℓ should be sampled for the toxicity tests and 1.5 ℓ for the toxicant analyses. When a resource has been classified it may be possible to reduce these volumes to perform only those toxicity tests relevant to the specific classification (*e.g.* sub-lethality tests need not be done for ecological category 2).

If a single laboratory is being used for both toxicity tests and toxicant analyses then samples need not be preserved, though samples must reach the laboratory within 24 hours. If different laboratories are being used, samples to be analysed for organic toxicants could be preserved with mercuric chloride if it is more convenient or cheaper to transport them to the organic laboratory within four days, instead of within 24 hours. However, it is preferable that no preservative is present at all during sampling to avoid contamination of samples intended

for toxicity tests. Therefore, if possible, no samples should be preserved and all should reach both laboratories within 24 hours. Samples should be transported well covered in ice (at 4ºC, not frozen), in a dark insulated container.

Analysis

Samples must be stored in the laboratory at 4ºC in the dark (not frozen). For all tests and analyses, except the algal growth inhibition test, unfiltered samples should be used. Some particulate losses will also occur when samples are prepared for the yeast test. For fish and *Daphnia* tests, samples can only be stored for one day. For algae, they must be filtered immediately and tested within three days. Samples for the yeast test should be extracted within three days and tested within the next three days. Preserved samples for organic toxicant analyses must be extracted with seven days and analysed within 14 days. Unpreserved samples must be extracted within a day and analysed within another day.

The recommended toxicity test methods are indicated in Tables 1-4 above. The organic toxicants should preferably be analysed using a Gas Chromatograph – Mass Spectrometer (GC-MS) though other detectors may also be used (electron capture, nitrogen phosphorous for triazines, or a flame photometer).

Data management

Data management involves a wide range of activities from properly registering the monitoring programme (monitoring points, monitoring frequency, etc.), managing sampling (printing schedules for monitors and laboratories, printing sample tags, etc.), receipt of analytical results (measured either in a laboratory or on-site), to capturing these results on the central database Water Management System (WMS) and making them available for subsequent processing (*e.g.* reporting).

Registration of the programme and individual monitoring points on WMS is done using standard application forms that can be found on the WMS website.

If the laboratory at D:RQS is used for all analyses, then data can be captured directly onto WMS. However, if direct data capture onto WMS from remote laboratories is not yet fully operational, a spreadsheet should be used to capture and transmit the data as an Email attachment. Emphasis should be on minimising the number of times data are captured manually.

Data retrieval will typically be achieved by exporting from WMS in delimited ASCII format. This facilitates simple importing into a variety of other software packages, such as the NTMP data assessment spreadsheet. Regular quality control checks should also be done, especially to ensure samples are being taken and that they are being analysed.

Criteria and guidelines

For a ecological category 1 (or a water use category 1) the water must not show any toxicity of any kind. For category 2, the water must not show any lethality (short- or long-term), although some sub-lethality may be observed. Although the toxicities are measured quantitatively (typically as a percentage effect) and recorded as such, for purposes of testing the null hypotheses no distinction is made between different levels of lethality or sub-lethality. For example, 20% fish lethality would place that water resource in a category 3 state, just as 100% lethality would.

For the toxicants, numerical guidelines should be used to establish the present state of the water resource. If a resource is not yet classified the most stringent guideline should be used for each toxicant (equivalent to the ecological category 1 or water use category 1).

Assessment and reporting

An important issue throughout all data assessment and reporting procedures is to ensure that results are not misinterpreted. Attention should also be paid to the likely causes and consequences of false negative and false positive results. It must be ensured that assessments support decision making that is genuinely informed. This includes making explicit statements in reports that ensure that results are properly interpreted. The following are some examples when this is necessary:

- When a detection limit is above a guideline value.
- To ensure that the bias on national coverage caused by the priority area approach is understood.
- Why individual monitoring points were chosen (*e.g.* what catchment, river reach, etc. they represent).
- If a toxicant is detected that does not occur naturally, this in itself is useful information (irrespective of guidelines).
- The individual guidelines themselves (for ecological category) should be interpreted at face value (as "trigger values"), not in terms of percentage of species apparently protected.
- Attempts should not be made to rationalise toxicant and toxicity results because they are based on fundamentally different approaches.

Ideally results should be presented both verbally and in written reports. Verbal presentation provides good opportunities for obtaining first-hand feedback from target users. This feedback (and any obtained on the written reports) should inform future periodic review of the NTMP. Written reports should present results in spatial formats, like maps, that are simple to interpret.

Quality assurance and quality control

It is recommended that the ISO 9001:2000 [SABS, 2000] quality management system be applied to the NTMP. The principles that underpin this system are customer focus, leadership, involvement of people, process approach, system approach to management, continual improvement, factual approach to decision making, and mutually beneficial supplier relationships.

Continual improvement is a particularly critical principle and is based on the cyclical plan, implement, check and review.

Achieving the NTMP objectives is the overall objective of quality assurance and quality control. Each aspect of the objectives should be periodically examined to ensure it is being achieved. If not, corrective actions should be imposed.

Creating positive attitudes and pride in all role players can be a powerful quality assurance tool. It can also be relatively inexpensive to implement. Simple mechanisms like communicating small successes and introducing rewards for work well done can facilitate motivation in those involved.

In respect of sampling and sample transport, a suitably qualified person can accompany the sampler/monitor on his/her rounds once a year. Sampling and sample transport procedures should be observed. If problems are evident they can be corrected immediately. In respect

of analytical laboratories, it is recommended that formal accreditation not be given a high priority until the NTMP becomes established and a basic level of nationwide analytical capacity has been created.

Staff continuity can create significant quality problems, sometimes even resulting in missing data. A simple precautionary strategy is proposed to ensure that there is a "backup" person who can take over from the primary person by the next time that particular task needs to be performed. If this precautionary approach is too onerous, then a simple risk-based calculation provides a way of quantifying the risks of quality problems arising from critical staff suddenly being unavailable. In this way, under some circumstances it may be acceptable to simply wait until a potential problem arises. The extra costs of training backup people can then be minimised by only focusing on those where the risk of quality problems are high.

The overall cost-effectiveness of the chosen QA and QC procedures should be reviewed initially on an annual basis.

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ABBREVIATIONS

AOAC	Association of Official Agricultural Chemists
CAS	Chemical Abstracts Substance
CMA	Catchment Management Agency
CPRG	Yellow chlorophenol red-ß-D-galactopyranosid
CSIR	Council for Scientific and Industrial Research
DCM	Dichloromethane
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (the insecticide)
D:RQS	Directorate: Resource Quality Services (DWAF)
DEEEP	Direct Estimation of Ecological Effect Potential
DO	Dissolved Oxygen
DWAF	Department of Water Affairs and Forestry
EC	Electron Capture
ECD	Electron Capture Detector
EDC	Endocrine Disrupting Compounds
FP	Flame Photometer (detector)
GC-MS	Gas Chromatograph – Mass Spectrometer
GIS	Geographical Information System
GLP	Good Laboratory Practice
ISO	International Standards Organisation
LC	Lethal Concentration
LIMS	Laboratory Information Management System
MFC	Membrane: Faecal Coliforms
MS	Mass spectrometer
MTBE	Methyl tertiary-butyl ether
NEMP	National Eutrophication Monitoring Programme
NOEL	No Observed Effect Level
NP	Nitrogen Phosphorous (detector)
NTMP	National Toxicity Monitoring Programme
NWA	National Water Act (36:1998)
OD	Optical Density
PCBs	Polychlorinated Biphenyls
POPs	Persistent Organic Pollutants
PTFE	Polytetrafluoroethylene
QA	Quality Assurance
QC	Quality Control
RIE	Relative Induction Efficiency
RP	Relative Potency
RQOs	Resource Quality Objectives
RWQOs	Resource Water Quality Objectives
SABS	South African Bureau of Standards
SANAS	South African National Accreditation System
SDC	Source Directed Controls
US EPA	United States Environmental Protection Agency
WMA	Water Management Area
WMS	Water Management System
WRC	Water Research Commission

GLOSSARY

Acute effect. See short-term effect.

Biotic. Of or pertaining to living organisms.

Biotoxicology: The qualitative and quantitative study of the adverse effects of chemical pollutants and other anthropogenic materials on organisms.

Carcinogenicity. The extent to which a substance can cause cancer.

Chemical pollutants. Chemicals dissolved or adsorbed on biotic or abiotic surfaces in water that can produce a toxic effect. These include metals or metal ions (*e.g.* lead, mercury, iron, manganese, etc.), inorganic chemicals (*e.g.* nitrate, ammonia, sulfate, fluoride, cyanide, etc.) and organic chemicals (*e.g.* phenols, petrochemicals, pesticides, steroids, algal toxins, etc.). Note that living organisms (*e.g.* faecal coliforms, viruses, parasites etc.) are excluded.

Chronic effect. See long-term effect.

Definitive test. An experimental technique that estimates the concentration of the toxicant at which a specified percentage or number of organisms exhibit a certain response. Typically reported as a toxicity endpoint, *e.g.* Lethal Concentration (LC), Effect Concentration (EC), Inhibition Concentration (IC), No Observed Effect Concentration (NOEC), etc.

Ecosystem. The total community of living organisms and their associated physical and chemical environment.

Endocrine disruption. The extent to which a chemical mimics, blocks or alters functions of natural hormones.

Fitness for use. A scientific judgement, involving objective evaluation of available evidence, of how suitable the quality of water is for its intended use or for protecting the health of aquatic ecosystems.

Fungicide. A pesticide compound specifically used to kill or control the growth of fungi.

Herbicide. A chemical pesticide designed to control or destroy plants, weeds or grasses.

Hydrocarbons. A very large group of chemical compounds composed only of carbon and hydrogen. The largest source of hydrocarbons is petroleum crude oil.

Hydrophilic. Having an affinity for water.

Hydrophobic. Repelling water.

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.

Insecticide. A pesticide compound specifically used to kill or control the growth of insects.

Lethality. The extent to which a toxicant can cause death by direct action.

Long-term effect. Any toxic effect (lethal or sublethal) that manifests *over a long period* (4 days or more) as a result of exposure to the toxicant. Also referred to as a chronic effect.

Long-term exposure. Exposure of the organism to the toxicant delivered in multiple events or continuously *over a long period*, generally weeks or more. Also referred to as chronic exposure.

Mutagenicity. The extent to which a substance can damage or change an organism's or cell's genetic material.

Organic. Composed of chemical compounds based on carbon chains or rings and also containing hydrogen with or without oxygen, nitrogen or other elements.

Persistence. Refers to the length of time a compound introduced to the environment, stays there.

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.

Petrochemicals. Chemicals made from petroleum or natural gas. Examples are ethylene, butadiene, most large-scale plastics and resins and petrochemical sulfur. Also called petroleum chemicals.

Petroleum products. Materials derived from petroleum, natural gas or asphalt deposits. Includes gasolines, diesel and heating fuels, liquefied petroleum gases (LPG and bugas), lubricants, waxes, greases, petroleum coke, petrochemicals and sulfur.

Pharmaceuticals. Drugs and medicinal compounds.

Pollutant. Any physical, chemical or biological object or substance that, when suspended, dissolved or adsorbed on biotic or abiotic surfaces in the water, causes pollution.

Pollution. Defined by the National Water Act (36:1998) as the direct or indirect alteration of the physical, chemical or biological properties of a water resource so as to make it (1) less fit for any beneficial use for which it may reasonably be expected to be used, or (2) harmful or potentially harmful to (a) the welfare, health or safety of human beings, (b) any aquatic or non-aquatic organisms, (c) the resource quality or (d) to property.

Reserve. Defined by the National Water Act as the quantity and quality of water required:

1. To satisfy basic human needs by securing a basic water supply, as prescribed under the Water Services Act (108:1997), for people who are now or who will in the reasonably near future, be (a) relying upon, (b) taking water from or (c) being supplied from, the relevant water source; and
2. To protect aquatic ecosystems in order to secure ecologically sustainable development and use of the relevant water resource.

Resource quality objectives (RQOs). Numeric or descriptive (narrative) goals for resource quality within which a water resource must be managed. These are given legal status by being published in a *Government Gazette*.

Rodenticide. A pesticide compound specifically used to kill or control the growth of rodents.

Screening test. A toxicity test performed on the water or test sample “as is”, *i.e.* without dilution. Typically reports a percentage effect or a yes/no result.

Short-term effect. Any toxic effect (lethal or sublethal) that manifests *within a short period* (4 days) as a result of exposure to the toxicant. Also referred to as an acute effect.

Short-term exposure. Exposure of the organism to the toxicant delivered in a single event or multiple events *over a short period*, generally hours or days. Also referred to as acute exposure.

Sub-lethality. The extent to which a toxicant is detrimental without causing death.

Surfactant. A soluble compound that reduces the surface tension of liquids, or reduces the interfacial tension between two liquids or a solid and a liquid.

Target organism. The biological system of concern that will potentially manifest one or more toxic effects.

Teratogenicity. The extent to which a substance is capable of causing the formation of congenital anomalies. (Thalidomide is a well-known teratogen.)

Test organism. The organism used in a toxicity test.

Toxicant. A chemical substance capable of exhibiting a toxic effect.

Toxic effect. A dose-related effect manifest as an impairment of the activity of the organism or the cellular or sub-cellular system. In the current context, these effects are also limited to those that can be detected, either currently or potentially, locally or

internationally, by a “toxicity test”, as defined here.

Toxicity. In the current context, the degree to which a water exhibits toxic effects.

Toxicity test. In the current context, a toxicity test is regarded as an experimental procedure that measures, under defined conditions in the laboratory or in the field, the toxic effects of chemical pollutants in water on a group of living organisms or a cellular or sub-cellular system.

Waste. Defined by the National Water Act (36:1998) as including any solid material or material that is suspended, dissolved or transported in water (including sediment) and which is spilled or deposited on land or into a water resource in such volume, composition or manner as to cause, or to be reasonably likely to cause, the water resource to be polluted.

Watercourse. Defined by the National Water Act as a river or spring, a natural channel in which water flows regularly or intermittently, a wetland, lake or dam into which, or from which, water flows and any collection of water that the Minister may declare to be a watercourse. Furthermore, reference to a watercourse includes, where relevant, its bed and banks.

Water Management Institution. Defined by the National Water Act (36:1998) as a catchment management agency, a water user association, a body responsible for international water management or any person who fulfils the functions of a water management institution in terms of the Act.

Water resource. Defined by the National Water Act (36:1998) as including a watercourse, surface water, estuary or aquifer.

CHAPTER 1: BACKGROUND

This chapter describes the purpose and scope of this implementation manual and why the NTMP is necessary.

1.1 PURPOSE

This prototype manual describes definitively how the National Toxicity Monitoring Programme (NTMP) for surface waters should be implemented in South Africa. It does this from a broad national perspective and at a detailed procedural level. The Department of Water Affairs and Forestry (DWAF or "the Department") is primarily responsible for implementation of the NTMP. However, this manual is aimed at all who will be involved, both inside and outside the Department.

This manual describes procedures for all important activities ranging from sampling through to assessment of data and reporting. It only provides reasons for procedures when an understanding of them is likely to improve the way the procedure is carried out. The reader is encouraged to consult the associated Record of Decision Report [DWAF, 2005a] for a detailed background on why certain design decisions were taken.

1.2 SCOPE

The design in this manual is intended for monitoring water quality status and trends on a national basis. The water quality refers to that in natural water resources, and specifically inland surface waters. Initially only the water column will be sampled. The intention is to extend this design in future to groundwater and estuaries and also to sampling sediments and biota. The national context gives a relatively low resolution and strategic perspective to the collection of data and their interpretation.

Objectives for the programme are defined in detail in Section 3.2.

1.3 BROADER FRAMEWORKS

Chapter 14 of the National Water Act (Act No. 36 of 1998) specifically requires the Minister to establish national monitoring systems that can assess, among other matters, the quality of water resources and the health of aquatic ecosystems. The national toxicity monitoring programme described in this manual (the NTMP) focuses on the water quality component of resource quality, which intimately determines the health of aquatic ecosystems.

The current design is aligned with the overarching "Strategic Framework for National Water Resource Quality Monitoring Programmes" [DWAF, 2004a], which provides general design guidelines and a framework for capacity building.

1.4 THE PROCESS

A needs assessment for the NTMP was performed prior to embarking on the current design phase [DWAF, 2003]. This identified objectives, target users, criteria for choosing toxicants, an approach for choosing toxicity tests, and various other general design considerations.

The 3-year design phase, culminating in this prototype implementation manual, involved a team of relevant specialists, frequent interaction with the Department and overall guidance from a Steering Committee. The latter consisted of members of the Department, including upper management, and other government departments.

A 2-year pilot testing phase will follow this design phase in which specific aspects of the current design will be tested. The present prototype manual will then be refined. The final manual will then be used as the basis for subsequent full-scale phased implementation throughout South Africa.

1.5 THE NEED FOR THE NTMP

1.5.1 Impacts on people

In 2002 the World Bank published the following document [Goldman and Tran, 2002]:

"Preventable Tragedies:
The Impact of Toxic Substances on the Poor in Developing Countries"

The messages in that document alone provide ample motivation for a national focus on toxicity and toxic substances in South Africa. The following series of statements is taken from this source.

- "Exposures to toxic chemicals can occur through contaminated food and water, skin absorption, inhalation, or transmission from mother to child across the placenta, and in breast milk."
- "The exposure is particularly dire for children, whose small bodies and early development make the effects all the more severe."
- “Poverty, development and potential exposure to toxicants are closely related”.
- "In many parts of the world, public health institutions focus on communicable diseases, like HIV infection in parts of Africa, enteric diseases from contaminated drinking water and food, and malaria. Increasingly, however, chronic diseases – caused in part by exposure to toxic substances – are emerging as problems in developing countries and among those in poverty."
- "The World Health Organisation has estimated an annual worldwide incidence of 3 million cases of acute, severe poisoning (including suicides), matched possibly by a much greater number of unreported cases of mild-to-moderate intoxication."

It is quite evident that the impacts of toxicants on people warrant concern and attention. Monitoring the degree to which toxicity and individual toxicants exist in water resources is one important component of establishing the extent to which these substances are a problem in South Africa.

1.5.2 Impacts on aquatic ecosystems

Inorganic toxicants (like heavy metals) and organic toxicants (like many pesticides, petroleum products, pharmaceuticals, etc.) can enter water resources and have devastating impacts on ecosystem integrity. The following summarises the critical ecological issues:

- Besides occasional immediate and highly visible impacts of accidental spills (like fish kills), many toxicants have more subtle, though no less serious, long-term impacts on aquatic biota.
- Some impacts, like endocrine disruption, manifest at extremely low concentrations of toxicants.
- The nature of many long-term impacts makes them difficult to detect and quantify.
- Some toxicants are highly resistant to degradation in the environment and may persist for decades.

- Some organic toxicants degrade rapidly in the environment, or are metabolised, to other chemicals that may also be toxic.
- Many organic toxicants and some heavy metals (like mercury) have an affinity for animal tissue (*e.g.* in fish) and sediments in water resources. They can gradually accumulate in these media to levels many thousands of times the original background levels.
- Contaminated animals can be eaten by other animals up the food chain (including humans).
- Contaminated sediments can be scoured during floods, mobilising trapped toxicants and increasing the risks of exposure downstream.
- Some toxicants, like the persistent organic pollutants (POPs) addressed in the Stockholm Convention (2001), are highly volatile. They can be transported vast distances through the atmosphere away from their original sources. POPs have even been found in the Arctic, Antarctic and remote Pacific islands [UNEP, 2002].

The complexity and the potential severity of the problems evident in the above further emphasise the necessity for programmes like the NTMP. However, the NTMP should be seen as only one of a suite of approaches that South Africa should adopt. These should include better characterisation of sources of toxic substances and associated risks and formulation of focused policy and legislation. These should focus on minimising risks to humans and ecosystems without unnecessarily compromising much needed socio-economic development.

1.5.3 Past and present monitoring in South Africa

Monitoring in South Africa relating to toxicants and toxicity has tended to be restricted to once-off surveys in selected water resources, short-term research projects within a relatively small geographical area,and local monitoring by bulk water suppliers. No nationwide initiative exists that can provide a consistent overall picture of the extent of toxicity-related problems.

The following are a few examples of past work or work in progress.

- Various research projects have surveyed the extent to which various heavy metals and pesticides occur in South African biota [*e.g.* Bouwman *et al.* (1990), Heath (1999), Heath and Claassen (1999) and Heath *et al.* (2004)]. These studies surveyed some highly industrialised catchments, and intensive agriculture and forestry catchments and where malaria control is practiced. They found that metals and pesticides in fish tissues were at levels that could cause human health risks under various exposure scenarios.
- A recent initiative funded by the Water Research Commission is focusing on endocrine disrupting compounds (EDCs) in the aquatic environment as well as the impacts on human reproductive potential. Sediments, fish and water are being monitored at a series of potential "hot spots" in South Africa for EDC activity.
- The University of North West has surveyed sediments from selected rivers, estuaries and harbours in South Africa for some persistent organic pollutants (POPs). Highest concentrations were found in industrialised areas [Vosloo and Bouwman, 2004].
- Rand Water performs a screening assessment of (POPs) in their raw water from the Vaal Dam every few years.

1.6 STRUCTURE OF THIS MANUAL

The manual is aimed at everyone involved in the NTMP. The following indicates the overall structure and contents:

- **Chapter 1: Background.** This chapter describes the purpose and scope of this implementation manual and why the NTMP is necessary.

- **Chapter 2: National Implementation.** This chapter details how to initialise the NTMP in a phased manner and sustain it in the long-term.

- **Chapter 3: Monitoring Framework ("Why", "What", "Where", & "When").** This chapter defines the NTMP objectives, what should be monitored, and where and when the monitoring should be done.

- **Chapter 4: Sampling and Analysis ("How").** This chapter describes how samples should be taken and how they should be analysed.

- **Chapter 5: Data Management and Reporting ("Data" & "Information").** This chapter describes how the monitoring data should be managed and how it should be assessed and reported.

- **Chapter 6: Quality Assurance and Quality Control ("How well").** This chapter describes how the overall quality of the NTMP can be assured.

- **Chapter 7: Roles and Responsibilities ("Who does what").** This chapter highlights the responsibilities of each role player in the NTMP.

- **Chapter 8: References.**

- **Appendix: Biotoxicology Overview.** This chapter describes a general overview of the field of biotoxicology (*i.e.* toxicants and their effects on organisms).

CHAPTER 2: NATIONAL IMPLEMENTATION

This chapter details how to initialise the NTMP in a phased manner and sustain it in the long-term.

2.1 GUIDING ISSUES

2.1.1 Introduction

This section describes some of the general issues that should guide the process of national implementation of the NTMP. They are of particular relevance to the programme manager.

2.1.2 National coordination

The primary role of the programme manager is to facilitate the nationwide implementation of the NTMP so that the national objectives (see Section 3.2) are achieved. The programme manager will need to be familiar with all aspects of toxicity monitoring and should be able to provide technical and managerial advice to the various role players. The programme manager must also ensure effective and efficient transfer of knowledge and experience gained by those already involved in the programme.

The programme manager should be the driving force behind initial and ongoing implementation on a national basis, playing a hands-on management role. A significant commitment is required from the programme manager (and therefore that person's superiors). The programme manager should be a 'doer' not a 'delegator'. In this way, the work of the programme manager will achieve more depth. National coordination is then likely to be more consistent and efficient since the execution of tasks will be less fragmented, because they are being done primarily by a single person.

2.1.3 Anticipating the problems

It is important that the implementation of the NTMP learn from the experiences of other national monitoring programmes. A number of existing national water quality programmes exist for surface waters. The respective programme managers should be consulted and problems encountered and methods by which they were solved should be discussed.

The River Health Programme (RHP) in particular encountered a number of problems in its endeavours at implementation on a national basis [Murray, 1999]. Although in some ways more complex than the NTMP, it is appropriate to take note of those problems and ensure that the implementation strategy of the NTMP is able to avoid or minimise them as much as possible.

At the highest level, lack of accountability and resource constraints were the two main driving forces of ineffective implementation in the early stages. Lack of accountability involved, among others, (a) a lack of clarity on responsibility for implementation, and (b) the lack of support from superiors. The NTMP programme manager should make responsibilities absolutely clear (see Chapter 7: Roles and Responsibilities) and ensure that superiors understand the value of the NTMP (see capacity building report [Murray *et al.*, 2005]). Resource constraints entailed (a) the high cost of consultants, (b) the lack of trained personnel and (b) time constraints. Some of these issues are also addressed in the capacity building report [Murray *et al.*, 2005].

It will be important to ensure that sufficient capacity (of appropriate quality) is available to meet the needs of the national programme as it is gradually phased in around the country. The programme manager should oversee and implement the capacity building plan. The rate at which new capacity needs to be created in South Africa will depend on strategic decisions made by senior management of the Department. In particular, the rate at which

the NTMP should be implemented (*i.e.* the number of new areas participating in the NTMP each year), will be a critical determining factor.

2.1.4 Demonstrating early successes

The RHP implemented a so-called 'Demonstration-for-Resource Allocation Spiral' model in its initial years of implementation. A similar approach should be adopted for the NTMP. In the case of the RHP, small-scale demonstration of the role of biomonitoring in water resource assessment and management led to recognition of its usefulness. This recognition, and the acceptance of a need for the technology, resulted in the further allocation of resources (financial and human). Basically, this approach assumes that demonstrating good results leads to increased support.

Initially, the programme manager should choose a few water management areas (WMAs) that can be used to demonstrate the usefulness of toxicity monitoring to other WMAs. However, a failed attempt could have very negative consequences and delay ultimate implementation significantly. Therefore, the NTMP must endeavour to 'get it right first time'. Accordingly, these initial areas must be carefully chosen.

2.1.5 Creating and maintaining awareness

Generic mechanisms (applicable nationwide) must be identified for conveying information on the NTMP to all interested parties. This can include the following:

- Development and regular updating of a web site dedicated to the NTMP.
- Presentations at appropriate local and international conferences and symposia.
- News releases.

The following are the concepts and issues that should be communicated:

- Demonstrating the usefulness of the NTMP to encourage recognition and acceptance (*i.e.* applying the 'Demonstration-for-Resource Allocation Spiral' model).
- Enabling potentially interested parties to identify whether they can benefit from the programme.
- Keeping readers up to date with implementation progress (*e.g.* what areas are currently included).
- Educating stakeholders about the causes and impacts of toxicity.
- Describing how one becomes involved in the national programme.
- Educating water users on how to avoid causing toxicity.

2.1.6 Managing participating organisations

2.1.6.1 Responsibilities

Meeting the objectives of the NTMP is, and will remain, the responsibility of the Department. However, regional implementation of the programme in water management areas is likely to be performed by the catchment management agencies (CMAs), acting as agents for the Department. Departments like Department of Environment Affairs and Tourism, Department of Agriculture and Department of Health are likely to have a significant interest in this monitoring programme and may well be able to contribute resources for its execution. Non-DWAF and non-CMA organisations may also be sub-contracted to perform specific tasks like sampling, sample transport, analysis and so on.

2.1.6.2 Top-down and bottom-up

The successful implementation of the NTMP will involve a careful combination of bottom-up and top-down approaches. The top-down approach will have its basis in the current legislation and the creation of an infrastructure to implement national information systems in accordance with the National Water Act (36:1998). The bottom-up approach involves identifying those regional and local concerned parties, sometimes individuals, who will themselves benefit from involvement in the NTMP.

2.1.6.3 Sustaining commitment

It is proposed that a 'contractual win-win reward' model be implemented in order to create and sustain an appropriate culture of commitment to the NTMP, particularly among the samplers. (More detail in this respect can be found in the research report associated with the National Eutrophication Monitoring Programme implementation manual [DWAF, 2002b]). This model has the following three primary components:

- Formal contracts with local agents. (a) For non-DWAF and non-CMA agents, this should be a binding contract in which the tasks to be performed are well-defined, including details on when, where and how they should be performed. Direct financial payments are then made on completion of the tasks. (b) For DWAF/CMA employees, these contracts should take the form of formal and clear modifications to their job descriptions (in the form of key performance areas). (c) The purpose of contractual agreements is to ensure, as far as is possible, that neither party (DWAF/CMA or the local agent) can unilaterally change the conditions of the contract. This ensures that local agents cannot simply change or terminate their involvement in the NTMP without negotiation when their local priorities change.
- 'Win-win' for DWAF/CMA and local agents. Local agents should be chosen who themselves see direct or at least indirect benefits from involvement in the programme. That is, they should preferably be local stakeholders with a vested interest in water quality of the water resource, for example either polluters or users. This further minimises the likelihood of a local agent not fulfilling the conditions of the contract.
- Reward commitment. A system should be considered that (a) rewards significant commitment to sampling and (b) creates a culture of commitment. This supplements the 'win-win' situation by further encouraging sound and frequent sampling.

2.2 PHASED IMPLEMENTATION PROCESS

2.2.1 Overview

A "national implementation process" is that series of actions required to set up and sustain a successful national monitoring programme throughout South Africa. Figure 2.1 shows the steps in the process [adapted from Murray *et al.*, 2004]. It assumes a programme manager has been appointed. The sections that follow refer to this figure and give details of the individual steps.

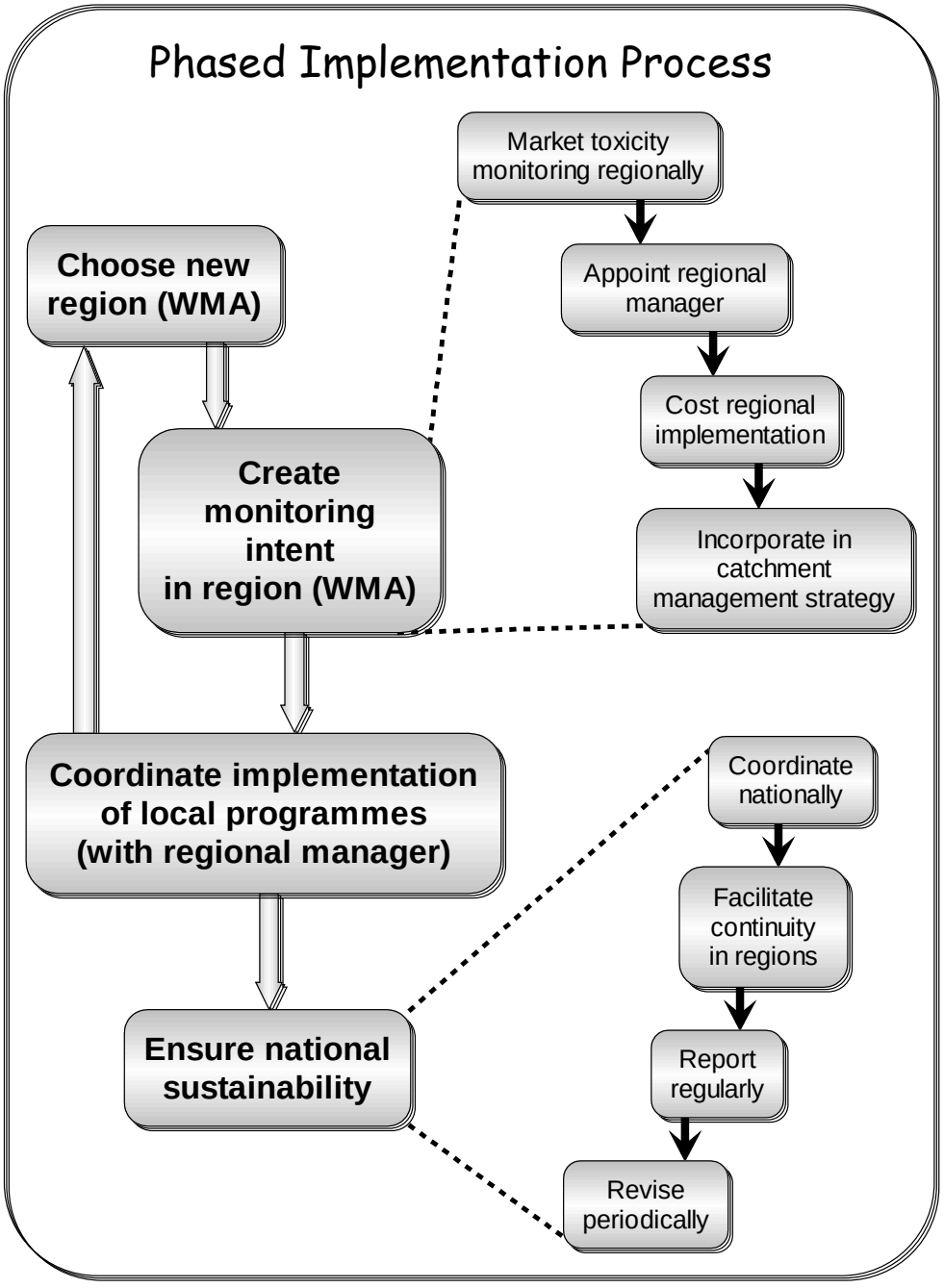


Figure 2.1. Summary of tasks in the national phased implementation process.

2.2.2 Choose new area (WMA)

As with other national monitoring programmes, the number of regions (water management areas) should be increased in a gradual and phased manner until adequate coverage of South Africa is achieved. Experience will dictate the practical rate at which regions can be included. Targets should be set by the programme manager for the number of regions included in five and ten year's time. The rate of inclusion should increase in later years as experience increases efficiency.

Initially, regions should be chosen in which toxicity is likely to occur and where there is either domestic use or important aquatic ecosystems exist and where there is good chance of successful implementation of the NTMP. The "priority area" approach described in Chapter 3: Monitoring Framework, Section 3.5, should be used to choose areas during the initialisation phase.

The rate of increase in monitoring should be closely aligned with the capacity building plan [Murray *et al.*, 2005].

2.2.3 Create monitoring intent in region (WMA)

2.2.3.1 Market toxicity monitoring regionally

Monitoring may already exist in some areas. In such cases, there already exists some degree of monitoring intent and therefore marketing of the NTMP should focus less on initialising monitoring and more on coordinating existing efforts.

The programme manager should visit the DWAF regional offices or catchment management agencies responsible for the chosen WMA. The primary purposes are to make them aware of the NTMP and create a local intent to become involved (if no monitoring exists). They should at least be given a copy of this implementation manual.

They should also be told the reasons why their WMA was chosen. A general introduction to the causes and effects of toxicity should be given (if necessary).

2.2.3.2 Appoint regional manager

A single person in the region should be appointed as the regional manager. This person would ideally be from the DWAF regional office (prior to catchment management agencies being established) or a member of the catchment management agency (if one exists). An assistant regional manager should also be appointed to ensure continuity during any absence of the regional manager.

The primary tasks of this person include managing the regional implementation process. This person will also be responsible for day-to-day management of the programme subsequently (*i.e.* once up and running).

2.2.3.3 Cost regional implementation

If there are any doubts regarding potential costs, the programme manager should collaborate with the regional manager and use the implementation costing model to obtain rough cost estimates for implementation in the region.

2.2.3.4 Incorporate in catchment management strategy

It is important that pollution management strategies, including those related to monitoring, be included in the overall catchment management strategy of the catchment management agency. The programme manager should work closely with the regional manager to ensure this happens. Management strategies of other existing regions can be adapted to suit the current region. A report is available that provides guidelines for developing the water quality management component of a catchment management strategy [DWAF 2001a.]

2.2.4 Coordinate implementation

The regional manager should work closely with the programme manager while coordinating initial implementation, especially concerning capacity creation. Lessons learned from other regions should be carefully considered and problems anticipated and avoided (see Section 2.1.3).

2.2.5 Ensure national sustainability

2.2.5.1 Coordinate nationally

The programme manager should address all the national implementation issues described above (see Section 2.1).

The 'coordination' role has two primary aims. The first aim is to enthusiastically drive the NTMP at all levels, but particularly from a national perspective. The second aim is to ensure that an adequate level of quality assurance is achieved so that the national objectives are met (see Chapter 6: Quality Assurance and Quality Control).

2.2.5.2 Facilitate continuity in regions

Monitoring programmes should be designed to be as self-sustainable as possible. However, in the initial years, active engagement by the regional manager is likely to be significant. This means that there is likely to be a significant reliance on the regional manager initially. Therefore, a sudden resignation (for example) of a regional manager may have serious consequences for the continuity of the NTMP. The programme manager must ensure that in such a case, continuity is maintained. This could be achieved by the early appointment of an assistant regional manager. (See Chapter 6: Quality Assurance and Quality Control.)

2.2.5.3 Report regularly

Information contained in any national monitoring system established in terms of the Water Act must be made available in accordance with the Access to Information Act (Act No. 2 of 2000). Raw data can be made available via access to Water Management System (WMS) at Directorate: Resource Quality Services. Information should also be provided in the form of the annual reports. These reports should be presented in a format appropriate to the requirements of the intended users. (See Chapter 5: Data Management and Reporting.)

2.2.5.4 Revise periodically

Best practices, technologies, objectives and relative priorities change all the time. It is an essential component of any monitoring programme that the overall programme be carefully revised from time to time. The actual time between revisions can be left to the discretion of the programme manager. However, a period of five years is appropriate initially.

The revision should be comprehensive and consider the appropriateness of all aspects of the monitoring programme design, including the following:

- *Extent to which objectives are actually being achieved*. This is the most important issue. In particular, the main target users of the reports being produced by the programme should be consulted. It should be established whether they perceive true usefulness. If not, suggestions should be elicited regarding what they would like to see in future.
- *Objectives of the programme*. If no longer entirely relevant, they should be changed to ensure that they are properly aligned within the water resource management context of the time.
- *Monitoring variables*. New research, either local or international, may suggest variables with improved characteristics that allow the objectives of the programmes to be achieved either more accurately, more efficiently or more cost-effectively. These new variables should be considered carefully.
- *Monitoring frequency*. Being able to decrease the number of samples per year in some areas without significant loss of information may lead to significant cost savings. On the other hand, numbers of samples per year may need to be increased in areas in which objectives are not being met.
- *Analytical and sample preparation techniques*. New analytical techniques may be able to deliver results more cheaply.
- *Data management protocols*. The efficiency with which the monitoring data are transmitted, stored and retrieved for reporting purposes should be assessed. Improvements should be implemented where necessary.
- *Quality assurance and quality control methods*. These need to be carefully examined to ensure that the monitoring data are of an appropriate quality and that all quality assurance activities are appropriately focussed on the real requirements of the programme.
- *Data assessment and reporting protocols*. New methods of conveying the results of the monitoring programme in more appropriate ways to the intended target audience should always be considered.

CHAPTER 3: MONITORING FRAMEWORK ("WHY", "WHAT", "WHERE", & "WHEN")

This chapter defines the NTMP objectives, what should be monitored, and where and when the monitoring should be done.

3.1 INTRODUCTION

This chapter describes the overall framework of the NTMP. First, it defines the objectives. It then provides the conceptual framework of the envisaged resource classification framework, describing how the NTMP can be aligned with this initiative. Linked to this, specific null hypotheses are proposed upon which the detailed design is anchored. Specific details, such as what should be measured, and where and when the monitoring should be done, are then described.

3.2 NATIONAL OBJECTIVES ("WHY")

3.2.1 Definition

The National Toxicity Monitoring programme is primarily an initiative (and thus the responsibility) of the Department of Water Affairs and Forestry. It is a "status and trends" monitoring programme, the design of which is described in this manual.

The most generic expression of the highest level national management needs is provided by the National Water Act (Act No. 36 of 1998). Monitoring addresses certain aspects of the "information management" function of water resource management. This is described as "managing the monitoring, collection, storage and assessment of water resources, social, economic and institutional data and information required, as a support to the other water resource management functions" [DWAF 2001b]. These other functions include policy and strategy, water use regulation, physical implementation, institutional support and auditing.

At a national level, government departments such as the Department need to have a national picture of the degree to which toxicity in inland surface waters is a problem and how this is changing over time (see Section 1.5). Accordingly, the NTMP has the following specific objectives:

National Toxicity Monitoring Programme
DWAF National Objectives

To measure, assess and report on a regular basis
on the status and trends of the nature and extent of,

first, the potential for toxic effects to selected organisms, and,
secondly, selected potentially toxic substances
in South African inland surface water resources

in a manner that will
(A) support strategic management decisions
in the context of (1) fitness for use of those water resources
and (2) aquatic ecosystem integrity, and
(B) be mindful of financial and capacity constraints, yet,
be soundly scientific.

The objectives of a monitoring programme define the reasons for the existence of that programme. Importantly, they also provide the primary statement by which the success of the monitoring programme will ultimately be assessed.

3.2.2 Explanation of terms

It is important that the objectives are clearly understood because they will affect decisions made during initialisation, execution and periodic review of the programme. To ensure a common understanding of the objectives, the meanings of the various terms are summarised as follows.

***National**:* The use of the word "national" in the title of this monitoring programme refers to a number of contexts.

First, the primary responsibility for the monitoring programme lies with the Department, a national government department.

Secondly, as a national department it has various international obligations relating to the following:

- South Africa is signatory to various international agreements and conventions.
- Participation in global monitoring programmes.

Typically descriptions of the "state of the environment" at national level are required for the above contexts.

Thirdly, the Department also recognises various national responsibilities. These include the following.

- A responsibility to keep abreast of international trends in emerging problems. Persistent organic pollutants (POPs) and endocrine disruption are two examples. A national monitoring programme can raise "red flags" when water quality problems are detected, typically triggering action (like more detailed monitoring) at regional level. This "early warning system" is therefore seen to be large in spatial scale (*i.e.* regional and national) and in time (like annual).
- Creation of monitoring capacity upon which further region-specific capacity creation can be based when catchment management agencies become operational.

***Measure**:* This means "perform an experimental measurement of some property of the water resource". In the current context this measurement might be the concentration of a specific toxicant (or class of toxicants), or simply their presence or absence. It might also mean a measurement of the degree of toxicity using a "toxicity test". Such measurements will comprise the raw data of the monitoring programme.

***Assess**:* This means "add value to the raw data by providing information based on that raw data and the way that the water needs to be used or other site-specific issues". A common and simple mechanism of assessment of monitoring data is comparison of a measurement with a guideline value, if one exists. Such guidelines are typically specific to the kind of water use.

***Report**:* Monitoring data must never be collected just for the sake of having data. The data, and their assessment, must always be reported to well-defined target users in an appropriate format at appropriate intervals.

***On a regular basis**:* Monitoring is not a once-off activity. The measurements, assessments and reporting should be done at regular intervals appropriate to the requirements of the target users. In the current context, the temporal scale is annual. This means that reports must be prepared and submitted annually to the target users. These reports are usually based on data collected at an appropriate frequency throughout the year.

***Status**:* This refers to the current situation relating to the nature and extent of the problem. Since the temporal scale is annual, this refers to the current year.

***Trends**:* These are the significant changes in the status from one reporting period (*i.e.* year) to the next, or shorter period if necessary to meet the monitoring objectives.

***Nature**:* This word refers to the type of problem.

For toxic effects, nature refers to the following:

- The kind of toxic effect (*e.g.* fish lethality or algal growth inhibition).

For toxicants, nature refers to the following:

- Classes (types) of the toxicants (*e.g.* pesticides, heavy metals), or
- Individual toxicants (*e.g.* DDT, mercury).

For both toxicants and toxic effects, if guidelines exist then a further characterisation of the "nature" of the problem (or potential problems) may be possible, depending on the exact nature of the guidelines.

Note that 'nature' does not include establishing the source of the toxicants (*i.e.* who or what activity is causing the problem). This would require a specialised monitoring design that is not seen as part of this national programme. However, when it is possible to make scientifically sound statements regarding actual or potential sources (*e.g.* distinguishing between natural and anthropogenic sources) then this can be done in the annual assessment report.

***Extent**:* This word refers to the degree or severity of the problem.

In the first instance, extent refers to the *spatial* extent (*i.e.* the areas that are affected).

For toxicants, the extent also refers to the concentration of the toxicant.

For toxic effects, the extent refers to the degree of toxic effect (like percentage effect).

For both toxicants and toxic effects, if guidelines exist then a further characterisation of the "extent" (*i.e.* severity) of the problem (or potential problem) may be possible depending on the exact nature of the guidelines.

***The potential for toxic effects**:* This phrase refers to the following two contexts:

- First, an actual toxic effect can be reported. This refers to a specific observation (measurement) of the impairment of activity of a selected organism, cellular or sub-

cellular system (the "test organism or system"). This would often be reported as a percentage effect, referring to that organism, cellular or sub-cellular system.

- The "*potential* for toxic effects" refers to extrapolating the above measurement to potential toxic effects to an organism or system *other than the one tested* (the "target organism or system"). This "extrapolation" to a different organism or system should only be done when it is scientifically sound (see below). That is, it should have been demonstrated experimentally that an observed effect in the test organism or system is correlated with an effect in the other organism or system. For example, toxic effects manifest in water flea (*Daphnia*) should not simply be interpreted as meaning that humans will show similar responses. ("Extrapolating" directly from water flea to humans is inappropriate.) However, a toxicity test using mammalian cells may be appropriate if humans are regarded as a critical target organism.

***Selected organisms**:* This refers to a suite of organisms specially selected for this national monitoring programme according to particular criteria. For more information on these criteria see the associated Record of Decision report [DWAF, 2005a].

***Potentially toxic substances**:* This phrase is used deliberately to allow for inclusion of toxicants known to have toxic effects as well as those that are only suspected (but not proven) to have toxic effects. The term "toxic substance" is synonymous with "toxicant".

***South African inland surface water resources**:* Although ultimately all water resources covered by the National Water Act will be included, this particular manual addresses inland surface waters (typically fresh waters) only.

***Support strategic management decisions**:* The ultimate objective of the monitoring is to support informed decision making by those responsible for management of water resources. In particular, *strategic* decisions are defined as those that are large in scale, both spatially and temporally. A large spatial scale refers to regional (water management area) and national scales. A large temporal scale refers to decisions that have implications over periods of a year or more.

In the context of fitness for use of those water resources and aquatic ecosystem integrity: This issue, in effect, refers to a core mandate of the Department. The Department is the public trustee of the nation's water resources and must ensure their sustainable fitness for use. Fitness for use of water resources is achieved through two main initiatives:

- *Resource Directed Measures*. These comprise the resource management class, the associated resource quality objectives and the Reserve. The class must capture the most desirable long-term balance between (a) the extent to which a water resource is protected (reflected in the aquatic ecosystem integrity) and (b) the way in which, and extent to which, it is used for socio-economic development. The resource quality objectives are management objectives that define the ranges of various properties of the resource associated with the management class designated for a particular resource. The Reserve is the quantity and quality of water required to (a) satisfy basic human needs and (b) protect aquatic ecosystems.
- *Source Directed Controls*. These measures focus on managing sources of pollution that impact on water resources and are strongly influenced by the resource directed measures in place. Specifically, in the current context, they aim to ensure that such measures ultimately allow resource quality objectives to be either maintained or ultimately achieved by gradual improvement.

The above-mentioned international and national responsibilities (relating to emerging problems and capacity creation) are contexts in which higher-level decision-making is required (that this programme will support). However, such lofty goals have the danger of being somewhat vague and being of little or no specific use at lower levels of water resource management. In order to ensure that this national programme is practically anchored at catchment level, a formal alignment is established with the resource classification system (see Section 3.3). This ensures that the NTMP design is explicitly "in the context of fitness for use of those water resources and aquatic ecosystem integrity".

In summary, the ultimate management objectives supported by the NTMP involve addressing national and international responsibilities while simultaneously taking account of how water resources are managed at catchment level.

***Mindful of financial and capacity constraints**:* Water quality monitoring is expensive. Monitoring toxicity is particularly so. It is therefore important that the monitoring is cost-effective. This is in respect of both financial resources and the existence and creation of the necessary capacity to perform all the required tasks (like sampling, analysis, database management, reporting, etc.). For detailed information on financial issues and a capacity building plan see Murray *et al.* (2005).

***Soundly scientific**:* It is essential that the monitoring is based on sound science. Frequently, this may apparently manifest itself as a conflicting requirement with being mindful of financial and capacity constraints. However, this need not be so. If the "scientifically ideal" monitoring design cannot be achieved, this must simply be reflected in the reported assessment (unless the data are obviously totally inadequate). For example, fewer data may mean assessments are more uncertain. As long as this increased uncertainty is acknowledged and properly reported, the user of the information is still in a position to make an informed decision, albeit with greater risk.

Both the design of the monitoring programme (described in this manual) and its implementation will need to be scientifically sound. The scientific soundness of the above objectives refers more to the implementation. In essence this may mean little more than not deviating from the design. However, tasks like choosing monitoring points and, in particular, assessing the data will require decisions to be made before and during implementation over which the design manual can only provide general guidance. It is at these times that special care should be exercised so that assessments are made that are accurate and absolutely defensible. Indeed, it may be useful to imagine that the assessment might need to be defended in court. This may provide a useful incentive for it being scientific.

3.2.3 Exclusions

The following are explicitly excluded:

- It is not the intention of the NTMP to identify sources of pollution. However, it is the intention of the NTMP to highlight local resources ("priority areas") in which more detailed studies may be necessary to either confirm the existence of a problem or isolate the causes of pollution. However, these studies are not within the mandate of the NTMP.
- It is not the intention that the NTMP report directly to the general public. The target users are those directly involved with water quality management.
- It is not the intention that the NTMP monitor explicitly for accidental spills, nor their subsequent effects.

3.3 RESOURCE CLASSIFICATION

3.3.1 Background

The Minister is required to develop a water resource classification system in order to determine the class and associated resource quality objectives for all significant water resources (Chapter 3 of the National Water Act (36:1998)). Furthermore, all subsequent water resource management must give effect to this class and the resource quality objectives. In essence, the class will be the overarching concept driving all water resource management and intimately linked to the catchment vision. It is important that a major initiative, like a national status and trends water quality monitoring programme, be aligned with this concept.

Although the classification system is still being developed, its general basis has been suggested in the National Water Resource Strategy [DWAF, 2004b]. Three management classes, representing the desired future state of the water resource, may be adopted, namely (a) Natural, (b) Moderately used / impacted, and (c) Heavily used / impacted. The present state of the water resource may be any one of these, or it may be unacceptably degraded. The latter state is regarded as unsustainable and the future desired state (*i.e.* the management class) will be set to heavily used / impacted for such resources. The resource will be rehabilitated until this state is achieved.

Importantly, social and economic considerations will also be included in determining the class to ensure that an appropriate balance between use and protection is achieved. Classifying a resource is equivalent to the stakeholders saying that they wish the resource to (a) maintain a certain degree of ecosystem integrity and (b) enable the water to be used for certain well-defined uses. The latter may vary from recreational, through industrial to various agricultural uses (like livestock watering, aquaculture, irrigation, etc.) and domestic use. The degree of ecosystem integrity and the nature of the uses must necessarily be compatible. For example, if the water is intended to be used for a purpose that inevitably impacts negatively on ecosystem integrity, it may be agreed that the latter may be sacrificed somewhat for the greater socio-economic advantages of the intended use.

It is these concepts that ultimately make the classification and the Reserve the first line of defence against unsustainable development.

3.3.2 Ecosystem integrity and water use category

In order to align the NTMP with what is envisaged for the classification system, it is assumed here that the ecosystem integrity and the way the water will be used will be considered separately but in a way in which they remain compatible. For example, for purposes of the NTMP, the following correspondence is used between the class and the two types of categories:

Table 3.1. Conceptual correspondence of NTMP categories with management classes.

Management class	Ecological category	Water use category
Natural	1	1
Moderately used/impacted	2	2
Heavily used/impacted		
Unacceptably degraded*	3	3

* This is strictly not a management class (*i.e.* a desired future state). However, it may refer to a present state.

It is the intention of the NTMP to monitor whether water resources are within their designated ecological and water use categories. The precise mapping of class to categories is specifically not addressed (nor reported) within the NTMP as this is an issue to be addressed by the classification system. Furthermore, a deliberate simplification is introduced by combining the categories corresponding to "moderately used/impacted" and "heavily used/impacted" classes into a single category (category 2). This simplifies the definition of guidelines that define boundaries between categories. Only the boundary between (a) categories 1 and 2 and (b) categories 2 and 3 need be defined. Hence, the determination of the ecological category requires only two guidelines as does the determination of the water use category.

The categories have also been deliberately numbered as 1, 2 and 3 to avoid deliberations on precise meanings of narrative descriptions (like Natural, Good, Fair, etc. or even Moderately used, etc.) which are strictly the domain of the classification system.

In summary, the degree to which the NTMP is "aligned" with the classification system is limited to the degree to which the three ecological and three water use categories (*viz.* 1, 2 and 3) can be "mapped" to future determinations of the class and the corresponding formal ecological and water use categories. The NTMP does not concern itself further with the classification system *per se*.

3.3.3 Null hypotheses

Three null hypotheses are used as the basis of the NTMP design, all implicitly referring to toxicity:

- The resource is not in an acceptable ecological category.
- The resource is not in an acceptable water use category.
- The current status is worse than last year.

These hypotheses provide a sound and simple basis for the NTMP design. If either of the first two hypotheses is true then management actions are required that must ultimately result in the desired state being attained. If false, then the water resource is in the desired state and management actions are required to ensure that state is maintained. The third hypothesis allows deteriorating situations to be detected.

3.3.4 Criteria

By proposing specific toxicity-related criteria for each ecological category and water use category, a system can be developed that provides a simple and sound basis for guidelines. These in turn will allow each hypothesis to be tested. Table 3.2 shows such criteria and Tables 3.3 and 3.4 show the recommended toxicity tests and how their results would be interpreted.

Table 3.2. The criteria upon which NTMP guidelines can be based for ecosystem integrity and fitness for use.

Ecological / Water use category	Criteria	Toxicity that may be observed
1	No toxicity of any kind	None
2	No lethality (short- or long-term)	Sub-lethality
3		Lethality

Table 3.3. Choice of toxicity tests and interpretation of results for ecosystem integrity.

		Is resource in an acceptable ecological category?	
Desired ecological category	**Recommended toxicity test**	**If toxicity detected**	**If toxicity NOT detected**
1	Sub-lethality	No	Yes
2	Lethality	No	Yes

Table 3.4. Choice of toxicity tests and interpretation of results for fitness for use.

		Is resource in an acceptable water use category?	
Desired water use category	**Recommended toxicity test**	**If toxicity detected**	**If toxicity NOT detected**
1	Sub-lethality	No	Yes
2	Lethality	No	Yes

3.3.5 Guidelines

The final aspect that enables the NTMP to be aligned with the classification system relates to specific guidelines. Guidelines are regarded here as those criteria against which observations are compared in order to test a null hypothesis.

In respect of toxicity measurements, the criteria in the above tables are these guidelines. For example, if the ecological category is designated as 1, then "no toxicity of any kind" should be observed (Table 3.2). The recommended test is, in effect, any test that measures sub-lethality (Table 3.3). Strictly it is being assumed here that for category 1, a lethality test is not necessary (since sub-lethality tests are typically more sensitive that lethality tests). However, a design decision guiding the choice of toxicity tests was that such tests should be able to detect both lethality and sub-lethality in the same experimental procedure (see Record of Decision Report [DWAF, 2005a]. In practice therefore, both tests are applied when the category is 1.

In respect of measured concentrations of toxicants, each "guideline" is a single number against which measured values can be compared to test the hypotheses. These numbers are given in Chapter 5: Data Management and Reporting since it is in the data assessment phase that they are actually used.

3.3.6 NTMP guidelines versus resource quality objectives

It is important that the relationship between NTMP guidelines and resource quality objectives is clearly understood.

Resource quality objectives are numeric or descriptive (narrative) goals for resource quality within which a water resource must be managed. Since they have significant legal status (they will be published in a *Government Gazette*), their determination will need to have a sound scientific basis and typically considerable measured data to substantiate them.

The NTMP guidelines and toxicity criteria for the three categories chosen for the NTMP are currently highly unlikely to have sufficiently sound datasets for becoming resource quality objectives. This will partly be due to the lack of monitoring data and partly because of their complex behaviour in the environment. This does not mean that the determination of NTMP guidelines should be any less scientific. It only means that the level of confidence will be much less than that associated with resource quality objectives.

It is because of these kinds of reasons that the NTMP should be seen as complementing the monitoring that will necessarily be carried out to determine the degree of compliance with formal resource quality objectives (so-called "performance monitoring"). Where formal performance monitoring is taking place, the NTMP can provide water resource managers with extra information on the degree to which the water quality in the resource is in the designated category (or not) from purely a toxicity perspective. In principle, however, it is conceivable that in future sufficient data may eventually be collected that may allow NTMP criteria and guidelines to be "promoted" to the level of resource quality objectives. However, this should not necessarily be regarded as an objective of the NTMP.

3.4 MONITORING VARIABLES ("WHAT")

3.4.1 Toxicity

Different kinds of toxicity to selected organisms have been chosen as the "toxicity variables" to be monitored for the NTMP. Specifically, it is the degree of toxicity in each case that is the "variable". The following tables define those that will be monitored. They were specifically chosen to cover three trophic levels in the environment. Other temporary variables may be included in the pilot phase. For example, the guppy test will be used while the zebra fish test is being phased in and the *Vibrio fisheri* test may also be performed.

Table 3.5. Toxicity monitoring variables relating to fish (using the semi-static zebra fish development test).

	**Lethality**	**Sub-lethality**
Short-term	% Zebra fish (*Brachydanio rerio*) embryo lethality (96 hours)	
Long-term	% Zebra fish (*Brachydanio rerio*) larval lethality (10 days)	% Effect on Zebra fish (*Brachydanio rerio*) hatching time

Table 3.6. Toxicity monitoring variables relating to invertebrates (using the *Daphnia pulex* reproduction test).

	**Lethality**	**Sub-lethality**
Short-term	% *Daphnia pulex* lethality (96 hours)	
Long-term	% *Daphnia pulex* lethality (21 days)	% Reproduction inhibition

Table 3.7. Toxicity monitoring variables relating to algae (using the algal 24-well microplate growth inhibition test).

	**Lethality**	**Sub-lethality**
Short-term		
Long-term		% Alga *Selenastrum capricornutum* Printz growth inhibition

Table 3.8. Toxicity monitoring variables relating to humans (using the recombinant yeast (hER) test).

	**Lethality**	**Sub-lethality**
Short-term		
Long-term		Recombinant (hER) yeast screen

3.4.2 Toxicants

The concentrations of a series of selected toxicants have been chosen as the "toxicant variables" for the NTMP. They are as follows.

POPs relating to the Stockholm Convention

- Aldrin (CAS No. 309-00-2)
- Chlordane (CAS No. 57-74-9)
- DDT (CAS No. 50-29-3) and selected breakdown products (reported as total DDT):
    - DDD
    - DDE
- Dieldrin (CAS No. 60-57-1)
- Endrin (CAS No. 72-20-8)
- Heptachlor (CAS No. 76-44-8)
- Hexachlorobenzene (CAS No. 118-74-1)
- Mirex (CAS No. 2385-85-5)
- PCBs (the Arochlors 1221, 1232, 1248, and 1254 as commonly reported)
- Toxaphene (CAS No. 8001-35-2)

Other organic toxicants

- Endosulfan isomers (α- and β-) and the sulfate breakdown product:
    - α-endosulfan
    - β-endosulfan
    - Endosulfan sulfate
- Lindane (γ-BHC) and the following isomers:
    - α-BHC
    - β-BHC
    - δ-BHC
- Monocrotophos
- Three triazines:
    - Atrazine
    - Simazine
    - Terbutylazine

In future, new toxicants should only be added if they contribute significantly to achieving the NTMP objectives. Specific criteria are proposed in the Record of Decision Report [Section 5.6, DWAF, 2005a].

3.5 MONITORING POINT SELECTION ("WHERE")

3.5.1 Priority area approach

The first considerations relating to choosing where monitoring (sampling) will take place are those that are large in spatial scale (*e.g.* national, water management area, catchment). The core "macro" consideration in the NTMP is the so-called priority area (or "hot spot") approach to monitoring. The main factors that establish a priority area are:

- Toxicity or toxicants occur, and
- Sensitive water users are at risk, or
- Important aquatic ecosystems are at risk.

3.5.2 Classification system

Although the resource classification system (as defined in the National Water Act, Act No. 36 of 1998) is not yet finalised, there seems to be general consensus on the broad approach (see Section 3.3). It is specifically assumed here that catchment outflows will be important monitoring points for the classification system (though not necessarily the only monitoring points). Whatever monitoring points are ultimately chosen for the NTMP, these should ideally be consistent with the philosophy of the classification system. In other words, any approach used to select monitoring points for the NTMP must, first, place the objectives of the NTMP as the highest priority and, secondly, choose points that are located where information can be obtained about whether or not the water resource is in the designated ecological category and water use category.

3.5.3 Identifying potential priority areas

3.5.3.1 Prioritisation process

In order to increase the cost-effectiveness of the NTMP, potential high priority areas should be identified in which monitoring can begin. By focusing monitoring in the initial stages of full-scale implementation in these areas, there is an increased chance of facilitating protection of (a) people using water for domestic purposes and / or (b) important aquatic ecosystems *where it actually matters as soon as possible*.

It should be noted that prioritising catchments very accurately is not necessary. Accordingly, a very simple ranking system, based on the following factors, will be adequate:

- Degree of potential toxicity (T). This is the degree to which toxicity can be expected. (0 = little or none, 3 = very high.) This is a function of the following:
    - Number of potential polluters,
    - Likely nature of toxicants,
    - Likely fate of toxicants,
    - Toxicity of toxicants.
- Degree of domestic use (U). (0 = little or none, 3 = thousands of people potentially exposed.) This should be interpreted in the context of the number of people using water directly from a water resource with little or no formal treatment. Such a water use is inherently "sensitive" to the presence of toxicants. If resources have been classified in the catchment, and in particular the water use category for domestic use has been designated, then this can also be used as guidance (Category 1 = 3, Category 2 = 2, Category 3 = 1). Emphasis should be placed on surface waters. People relying on groundwater are not considered in this design.

- Degree of importance of ecosystems (E). (0 = not important, 3 = very sensitive, strategic importance, Ramsar site, World Heritage site, etc.) One approach could use the ecological category of any resource classification as guidance, ranking the categories as follows: Category 1: E = 3, Category 2: E = 2, Category 3: E = 1. A second alternative approach could be that developed by Kleynhans (1999) in which the "ecological important and sensitivity category" (EISC) can be determined. The designated EISC for the water resources in question should be mapped to a value for E as follows: Category "very high": E = 3, Category "high": E = 2.3, Category "moderate": E = 1.7, Category "low/marginal": E = 1.

The overall priority (P) of the catchment can then be simply calculated using the following formula:

$$P = T(U + E)$$

It should be noted that this can be applied at any spatial scale: water management area, primary catchments, secondary catchments, etc. It might also be noted that in risk terminology, T reflects the 'hazard' while T combined with U and E (to give P) reflect the risk (since the degree of use or the presence of important ecosystems represent the exposure to the hazard).

Hint: A useful mechanism for assigning relative rankings to the chosen catchments is to identify those with the highest ranking (the worst) and identify those with the lowest ranking (the best). Follow this by ranking the others relative to these.

Those with the highest calculated P value (the maximum value is 18) are the "high priority" catchments and should be considered first. If this is done starting with water management areas, this can be repeated for the individual sub-catchments (say down to quaternary) of the priority water management areas.

In general, problematic land uses that will determine the degree of toxicity include the following:

- Mining and chemical industries (from which various toxicants can enter water resources). These are typically point sources.
- Agricultural use (where pesticides are likely to be used). These will often be diffuse sources.
- Any area in which specific toxicants are known to be used. An example includes DDT which is only used in malaria areas.

It might also be noted that POPs are transported atmospherically over vast distances (*e.g.* they have been detected in the Antarctic). Therefore, POPs can potentially occur in all South African water resources. However, it is likely that concentrations in water resources are likely to be higher in the immediate vicinity of specific sources.

Some international sources and uses of some toxicants are given in Table 3.9. It should be noted that potential sources of many toxicants include not only where they may be used (or where they have been used in the past) but also where they are manufactured.

Table 3.9. International sources and uses of selected toxicants [Mörner *et al.*, 2002, UNEP, 2002].

Toxicant	Possible sources and uses
PCBs	Electrical equipment such as transformers, capacitors, circuit-breakers, voltage regulators, etc.
Aldrin	Against termites and other soil pests, termites attacking building materials, in grain storage, and vector control
Toxaphene	Control of insects in cotton and other crops
Chlordane	Against termites and other soil pests, termites attacking building materials
DDT	Control of medical and veterinary vectors, such as malaria-transmitting mosquitoes, plaque-transmitting fleas and trypanosomiasis-transmitting tsetse flies
Dieldrin	Control of locusts, termites, human disease vectors
Endrin	Formerly used against locusts and rodents. No current or recent uses known.
Heptachlor	Against termites and other soil pests, termites attacking building materials
HCB	Formerly used for seed treatment against fungal diseases, as well as for industrial purposes. No current or recent agricultural uses known.
Mirex	Against leaf-cutting ants, termites in buildings and outdoors, and also as a fire-retardant and for other industrial purposes

The points of outflow from priority secondary catchments should be chosen as monitoring points for the NTMP. Exact points should then be identified on the basis of the micro considerations.

3.5.3.2 Subjective and objective approaches

Two parallel approaches can be adopted to implement this above, depending on available resources. The formula above should be used in both cases.

Subjective approach

For this approach, it is adequate for the programme manager of the NTMP to meet with representatives of each DWAF regional office or catchment management agency in turn. These representatives should be responsible for existing water quality monitoring. Using their local knowledge a list of (say, secondary) catchments should be identified that are likely to be appropriate for toxicity monitoring (*i.e.* have potential polluters and have sensitive domestic users and/or important aquatic ecosystems). The resources available for monitoring will determine the number in this list. Catchments very unlikely to be problematic should be ignored. The above process should then be applied to this list. The group should reach consensus on the relative rankings.

Objective approach

The objective approach can, for example, be based on a formal GIS-based representation of each of the three factors:

- Land use maps should rank the degree to which land use is considered problematic on the above scale (0 to 3).
- Maps of domestic use should do the same. These should consider water resources abstracted for water treatment as well as those that may be used directly with little or no treatment.

- Finally, sensitive and important aquatic ecosystems can be ranked as indicated above.

These three maps can be overlaid to provide a map with the value of P calculated as above. High values of P will be the water resources that are most likely to be priority areas. Nevertheless, actual selection of these points should only occur after consultation with local water managers who should confirm that these "objectively" chosen areas do indeed make sense.

Again, bearing in mind that high accuracy in the final priority list is not necessary, the process of initialisation of the NTMP should not be unnecessarily delayed by the objective approach. Initially the subjective approach should be quite adequate for choosing areas in which to initialise the NTMP.

3.5.3.3 Monitoring points at priority catchment outflows

Once a final choice of monitoring points at catchment outflows has been made, a brief summary of the reasons for choosing each point should be recorded. These should appear in annual reports containing assessments of data from these points. This ensures that readers understand what spatial area (typically a catchment) is represented by each monitoring point. This serves the purpose of creating a connection between the single point being monitored and the area that it represents.

3.5.3.4 Monitoring points upstream of priority catchment outflows

It may also be necessary to identify other water resources upstream of the catchment outflows that should be monitored (in addition to the catchment outflow). This is because, in some catchments, the state of toxicity at the catchment outflow may not be a good reflection of the state elsewhere in the catchment (*i.e.* not representative of the catchment). For example, once released into the environment, persistent (non-biodegradable / conservative) toxicants can experience a wide range of fates, ranging from volatilising to the atmosphere to accumulating in sediments and biota. If monitoring the outflow only is adequate, then this catchment need not be considered further.

A simple process for identifying such potential monitoring points is illustrated in Figure 3.1. It should be carried out in close consultation with regional (or catchment management agency) representatives.

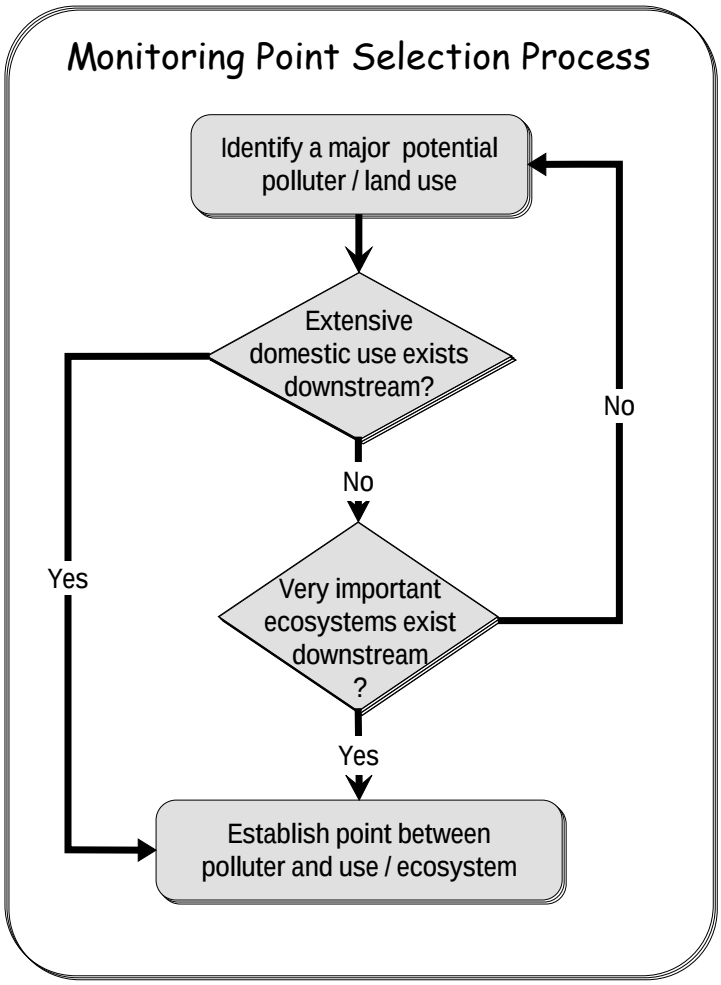


Figure 3.1. Simplified pollution source-related monitoring point selection process for the NTMP.

The process specifically establishes where toxicity may exist AND where there exists a potential risk to human health and/or aquatic ecosystem integrity. This should be done making good use of local knowledge of the area, as follows.

- Identify major potential polluters (land uses of concern). DWAF regional offices and catchment management agencies have a responsibility for monitoring pollution sources. The regional offices are therefore likely to be able to identify potential polluters relatively easily.
- Determine whether or not there are nearby downstream users that rely extensively on surface water either for bulk water supply or directly for their domestic use and that may be at risk should toxicity occur.
- Determine whether or not there are very important ecosystems downstream whose integrity may be at risk should toxicity occur.
- If either of the latter occurs, then determine locations in the water resource where toxicity or toxicant concentrations are likely to be maximised based on (a) the nature of the polluter, (b) the toxicants and (c) the location of downstream users or ecosystems that may be at risk. However, remember that it is not the explicit role of the NTMP to identify polluters. It is the role (actually the stated objective) to support strategic management decisions in the context of fitness for use and aquatic ecosystem integrity. Therefore monitoring where either (or both) of the latter is at risk will directly achieve the NTMP's objectives. However, the monitoring points should find a balance between maximising measured effects and being able to deduce

information about risks to human and ecosystem health. A point immediately upstream of the domestic users or ecosystem is therefore ideal (see Figure 3.2).

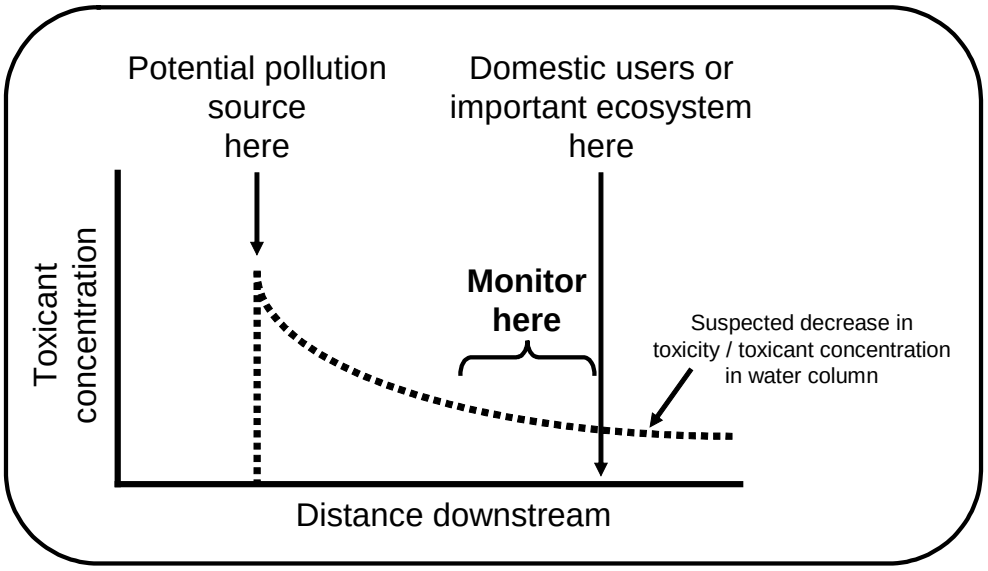


Figure 3.2. Schematic illustration of ideal monitoring point location that balances location of polluter and sensitive downstream domestic users and/or important ecosystems.

As above, clear reasons should be recorded for the choice of each upstream monitoring site that can appear in assessment reports.

3.5.3.5 Nature of the resource

The exact nature of the water resource that should be selected (*e.g.* deep, shallow, fast-flowing, slow-flowing, impoundments, etc.) is primarily determined by the factors mentioned above. Generally, it must be sufficiently representative of the chosen catchment. The outflow gives one aspect of "representativeness", and it also allows cumulative effects to be monitored.

Monitoring points upstream of catchment outflows (should any be chosen) would typically be in a significant river reach in a main watercourse or possibly one of its tributaries, or an impoundment. The strategic nature of the NTMP suggests that minor local types of water resource (like pools, riffles or minor tributaries) would probably not be appropriate.

3.5.3.6 Available financial resources

Available financial resources will be very important in determining the number of monitoring points. Once a series of potential points in any chosen water management area have been chosen they should be submitted to a preliminary costing exercise. Attempts should then be made to secure the necessary funding. Should there be any shortfall then the points with the highest priority ranking should be chosen first.

3.5.4 Site-specific considerations

Once a potential priority catchment has been identified and the approximate location of monitoring points identified (like the outflow or some upstream point), more local and site-specific factors must be considered in order to choose the exact locations. Many of these factors apply generically to any kind of monitoring.

3.5.4.1 Spatial correlation

Ideally, spatial correlation should not occur between samples taken at different points. This means that a sample at one monitoring point should not vary in composition in a way that can be predicted from the composition of a sample taken at the next closest point. If significant correlation occurs, resources are being wasted because the second point is not providing information that cannot be obtained from the first point. Furthermore, the data from different monitoring points may now be correlated and this should be borne in mind when selecting methods for data reduction and information generation.

The existence of correlation between monitoring points can only be formally established when sufficient data from the points are available. It is conceivable that this will only be necessary once full-scale implementation has begun. Due to the inevitable phased implementation at increasing numbers of points over the years, it is likely to be relatively easy to avoid spatial correlation initially (using common sense) by placing monitoring points at significant distances from one another. The emphasis will initially be on obtaining data for a water management area (WMA) as a whole. Completely different river systems are likely to be chosen to achieve this. Therefore, spatial correlation is not very likely (though should, nevertheless, be specifically borne in mind when choosing points at this time). However, in subsequent years as more and more points are added to each WMA to obtain better coverage, spatial correlation will naturally become increasingly likely. Therefore, an examination of spatial independence of information (which may include spatial correlation) should form part of the regular review of the NTMP.

3.5.4.2 Health and safety

The health and safety of people doing the sampling is extremely important. The following should be carefully considered.

- Potential danger from the water being sampled (the monitoring point is after all being chosen because there is potential toxicity). If necessary, gloves and protective boots should be worn.
- Potential danger from wild animals.
- Potential danger from local people.
- Potential for vehicle hijackings.
- Local physical hazards (like steep slopes).

If any of the above (or any other factor) is potentially problematic, appropriate steps should be taken to minimise or preferably avoid the risk, including choosing another monitoring point (if appropriate).

3.5.4.3 Accessibility

The monitoring point should be easily accessible to the person taking the sample. Valuable time and resources are wasted if this is not the case. Sampling is an expensive item in an overall monitoring programme. Considerable attention should be given to making it as efficient and cost-effective as possible.

3.5.4.4 Mixing zone

Attention must be given to the inevitable mixing zone that exists immediately downstream of an initial point of impact of an effluent discharge and even some non-point sources. Monitoring must take place well beyond the mixing zone to avoid unrepresentative samples being taken [Sanders *et al.*, 1983]. It must be ensured that the reach indicated as requiring monitoring in Figure 3.2 is located beyond this mixing zone.

3.5.4.5 Fate and transport of toxicants

The concentration of a toxicant in the environment depends on (a) the nature of the toxicant and various properties of it and (b) various properties of the aquatic environment in which it occurs (for more detail see Appendix: Biotoxicology, Section A.3).

These properties, and the presence and nature of sediments and biota, determine whether or not a particular toxicant prefers to dissolve in the water, accumulate in sediments or biota, volatilise into the atmosphere, bind to suspended particles, degrade to other chemicals, and so on.

Understanding the fate and transport of toxicants at this level is highly technical, demands much data and requires specialised modelling. It will usually suffice to familiarise oneself with general descriptions in the literature of the likely fate and modes of transport of individual toxicants. If such information is not readily available, the most important properties of a toxicant to take note of will be:

- Its persistence (resistance to degradation),
- The degree to which it accumulates in sediments or in biota, and
- The degree to which it might volatilise into the atmosphere.

Besides the fate and transport of toxicants being complex, the manifestation of toxicity is even more complex. This is determined by the nature of the organism and the nature and degree of exposure that it experiences (for more detail see Appendix: Biotoxicology, Section A.2).

It is important to acknowledge this complexity. However, it is not advisable to try to take such detail directly into account when determining the location of monitoring points unless specialist expertise is readily available. The Chemprop model (see Section 3.6.5) may in future be able to provide such information.

3.5.4.6 Existing monitoring points

The presence of monitoring points used for other monitoring programmes can be chosen in order to make use of existing sample collection capacity (or at least to share the costs of sampling with the other programmes). Indeed, this kind of consolidation with other programmes is essential in avoiding duplication of sampling rounds. Since such sampling usually comprises a significant proportion of the costs of any monitoring programme, consolidation can result in significant cost-savings. Outflows from secondary catchments at which the Department currently has registered monitoring points are likely to be adequate for the "catchment outflow" locations noted above.

However, it is absolutely imperative that the choice of such points is based on considerations relevant to the NTMP. This will ensure that achieving the NTMP's objectives is not compromised.

3.5.4.7 Proximity to laboratories

When a choice exists, and particularly in the initialisation stages in a new area, it may be preferable to choose monitoring points that minimise potential logistical problems until procedures become better established.

3.5.5 Monitoring point registration

Once chosen, each monitoring point must be formally registered on the WMS (see also Section 5.2.2). The following is required:

- Monitoring Point Name. (Text containing the monitor's reference, the name of the point and on what water feature it is situated.)
- Feature (water resource) on which monitoring point is located.
- Latitude and longitude.
- Reference number of 1:50 000 map on which point is located.
- An A4 copy of the 1:50 000 map showing the monitoring point.
- How the latitude and longitude were determined (from map or using a GPS).
- Datum of GPS instrument or map (Cape / Clarke 1880 / Hartebeesthoek94 / WGS84 or other).
- Quaternary drainage region.
- Detail of the organisation(s) responsible for monitoring.

3.6 MONITORING FREQUENCY ("WHEN")

3.6.1 Terminology

Note that since the unit of frequency is generally "events per unit time", *e.g.* monitoring rounds per year, the term "minimum frequency" strictly means the minimum number of events per unit time (or monitoring rounds per year), which in turn refers to the maximum time between events (or monitoring rounds). To summarise:

- Minimum frequency corresponds to the maximum time between monitoring events or the minimum number of samples per year.
- Maximum frequency corresponds to the minimum time between monitoring events or the maximum number of samples per year.

However, notwithstanding these definitions, in the interests of simplicity intended to avoid any confusion whatsoever, the terms minimum and maximum frequency will be not be used here. The following terms will be used instead:

- "Minimum number of samples per year" will be used instead of "minimum frequency".
- "Maximum number of samples per year" will be used instead of "maximum frequency".

Although the term "number of samples per year" suggests that such samples be collected at a fixed time interval (say, every two weeks) over the year, it is acknowledged that it is conceivable that sampling intervals could potentially be seasonal. However, since too few data are currently available, it not possible at this time to address this issue in any detail.

Generally speaking there are three critical factors that determine the optimum number of samples per year. These are discussed in the following sub-section.

3.6.2 Factors affecting number of samples per year

3.6.2.1 Achieving objectives

The most important factor determining the number of samples per year is the minimum amount of data required to achieve the objectives of the programme. To determine this it is necessary to quantify a direct relationship between the objectives and number of samples per year. This can be done if the objectives are expressed in terms of well-defined null hypotheses. Three null hypotheses have been defined (see Section 3.3.4). However, to establish the number of data points required to test any of these hypotheses, at least the following kinds of issues need to be addressed:

- In respect of the first two hypotheses, is the resource regarded as not being in an acceptable ecological category (or water use category) when:
    - Any single measurement is outside a corresponding guideline value, or
    - When say 5% of measurements in any one year are outside the guideline value?
- In respect of the last hypothesis, the number of data points required for each year will depend on the natural variation of the monitoring variables (caused by ecological factors like flow, temperature, etc.) and the manner in which toxicants may be entering the natural environment (constantly, seasonally, regularly, in random spikes, etc.). Typically, the greater the variation the more data will be required.

The first two hypotheses are intimately related to the envisaged classification system. Since this system has not yet been established, and since the NTMP should ideally be aligned with it, it is proposed for simplicity that a resource is regarded as not being acceptable if any single measurement is outside the corresponding guideline. When the classification system is established, this can be re-visited and revised if necessary.

In respect of the last hypothesis, since no data presently exist that allows the natural variability to be established, it is not possible to establish the minimum number of samples required per year. This can be done when sufficient data become available.

3.6.2.2 Avoiding temporal correlation

Data collected at regular intervals from a particular monitoring point may be "temporally correlated" if the sequential data values vary in a similar way. For example, if weekly sampling is being performed, the data from any particular week may vary similarly to that of the previous week. This is equivalent to saying that a particular week's data contain similar information to that of the previous week. This is not only a waste of resources but this can also affect the validity of statistical analyses based on those data (since independence between individual data points is often required).

The existence of temporal correlation can only be properly determined when real data are available for different monitoring points. Monitoring points will need to be analysed separately to establish a maximum number of samples per year required at each point to avoid temporal correlation. Until such time as the data are available, common sense should prevail (based on local knowledge of the behaviour of the water resources in question) to ensure as far as possible that correlation is avoided.

3.6.2.3 Available resources

Since the number of samples per year affects the costs of monitoring directly, the human and financial resources available may impose practical limits on this number.

As an example, limits on the number of samples per year may arise from the limitations associated with the maximum number of samples that can be processed per week (or per day) by samplers and analytical laboratories. Of course, a large number of samples per year may require an increase in such capacity but until it is created the number of samples per year may well be limited by this.

3.6.2.4 Summary

The above sub-sections noted three main factors that determine the optimum number of samples per year (see Figure 3.3). Naturally, the maximum number of samples per year that avoids temporal correlation may be greater or less than that that can be processed with available resources. The figure shows the former as less than the latter. The figure also assumes that the minimum number of samples per year required to achieve the programme's objectives is less than the available resources (otherwise the programme is, by definition, not feasible).

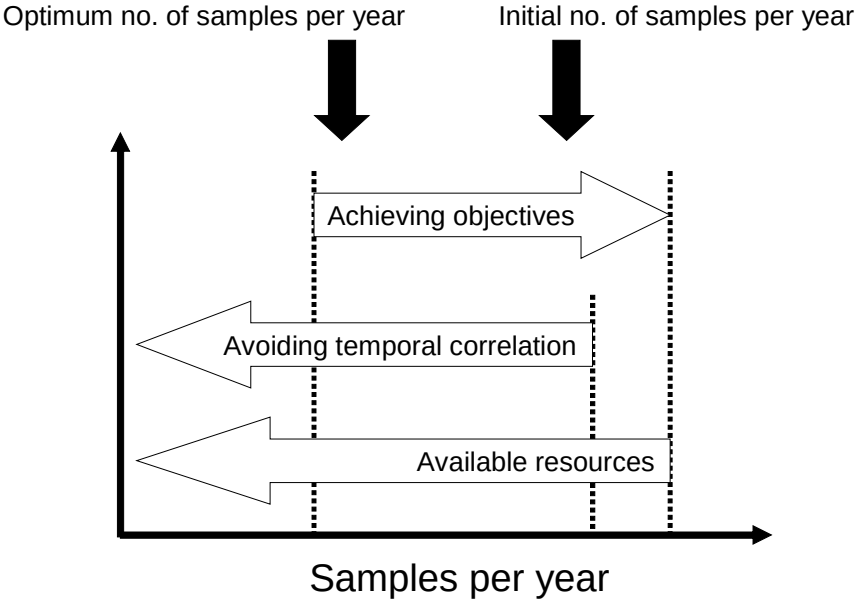


Figure 3.3. Schematic illustration of factors determining the optimum number of samples per year.

In essence, the number of samples per year must:

- Be sufficient to achieve the objectives of the programme in respect of supporting strategic decision-making,
- Be low enough to avoid temporal correlation, and
- Be within the ability of available resources to process.

It might also be noted that the objectives include being mindful of capacity and resource constraints. Therefore, the optimum number of samples would typically be the least number of samples per year that achieves the objectives.

When no data are available, then the initial number of samples per year should be as high as the available resources will allow (see Figure 3.3). Ideally this should also avoid temporal correlation but without data this may not be possible to ensure.

3.6.3 Proposed initial number of samples per year

To date very few local data are available on which a scientific determination of monitoring frequency can be based. It is therefore proposed that monitoring takes place at the highest monitoring frequency that is practically implementable using the human and financial resources available at as many different monitoring points as possible (preferably around 5). Sampling every two weeks, or even monthly, may be sufficient in a pilot phase over one hydrological cycle. This dataset should then be used to estimate an appropriate monitoring frequency.

3.6.4 Compromising on optimum numbers of samples per year

A number of circumstances can be envisaged in which the optimum number of samples per year may be compromised (*i.e.* a lesser number actually chosen).

As data become available, it may be found that different monitoring points require different numbers of samples per year. If they are similar in magnitude, it may be satisfactory to assume a single average number for all the NTMP monitoring points. This may compromise the information content at some monitoring points. (If there is a large range it may be necessary to consider different numbers of samples per year for different monitoring variables, or possibly type of monitoring point. However, the logistical, managerial and data assessment implications of such a decision would need to be carefully considered.)

Even once an optimum number of samples per year has been decided for a particular monitoring point, it may occur that external partners approached to perform sampling simply do not have the resources for that number of samples. If the shortfall cannot be addressed by the Department, a lesser number of samples may be accepted, applying the philosophy that "some data are better than no data".

Importantly, in any of the above scenarios, if an optimum number of samples is being compromised, the consequences of such a decision on the quality of information provided to water resource managers must be established (preferably quantified, if possible). In the interests informed decision-making, if any such compromise is made then the following must occur:

- The compromise on quality (even if not quantified), specifically relating to potentially not achieving the stated objectives of the programme, must be:
    - Explicitly acknowledged by all concerned, and
    - Explicitly stated in assessment reports.
- The decision-makers must accept responsibility for any potential increase in risks.

This strategy acknowledges current realities, is deliberately pragmatic and is a standard risk-based approach to management. Less than optimum sampling can increase risks. If this increased risk is acceptable to the manager, then it can be adopted.

3.6.5 Modelling

A project is underway at the present time to calibrate the ChemProp computer model [Schüürmann *et al.*, 1997] developed at the Umweltforschungszentrum (UFZ), or Centre for Environmental Research, in Germany under the leadership of Professor Gerrit Schüürmann. Should this model be considered adequate for local conditions, it may provide further information on optimum numbers of samples per year. However, it may be some years before this model can be used.

CHAPTER 4: SAMPLING & ANALYSIS ("HOW")

This chapter describes how samples should be taken and how they should be analysed.

4.1 INTRODUCTION

How to take samples and how to analyse and test them are the physical and technical aspects of the monitoring programme. It is critically important that well-trained people perform these tasks in the prescribed manner and at, or within, the specified times and quality standards. Inadequate attention to detail can directly compromise the quality of the data and hence the quality of information intended for water resource managers.

The associated research report should be consulted for more detail on the specific rationale behind each of the chosen procedures [DWAF, 2005a].

4.2 SAMPLING PROCEDURES

4.2.1 Sample management

Sample schedules and sample bottle tags will be provided by Directorate: Resource Quality Services (D:RQS) (see Section 5.2.3). These schedules can only be produced after monitoring points have been identified and registered on the Water Management System (WMS) (see Section 3.5).

4.2.2 Apparatus

- Sample bottle tags and waterproof pen
- *For toxicity tests*: Chemically clean and preferably sterile clear glass sample containers sufficient for a total of 8 ℓ per sample. Screw caps must be lined with PTFE Teflon.
- *For toxicants*: Chemically clean dark glass sample containers sufficient to contain 2.5 ℓ per sample. Screw caps must be lined with PTFE Teflon.
- Cooler bags/boxes and ice packs for keeping samples cold.

4.2.3 Grab sampling

Sampling protocols are outlined in various documents [IWQS, 1997; Meinhardt, 2004; Slabbert, 2004]. The ultimate quality of monitoring results is directly dependent on (a) obtaining samples that are representative of the water resource, (b) maintaining their integrity until tests or analyses can be performed, and (c) the training and experience of samplers/monitors.

When flowing water (*e.g.* in rivers) is sampled, samples should be taken from the water column as far away from the bank as can conveniently be reached from the bank. Stationary water should be avoided. However, in dry periods, large river pools can be sampled.

When sampling stationary water in impoundments or lakes, samples should ideally be taken away from the shore towards the middle of the water body. They should ideally not be taken next to the dam wall or the shore because this water may not be representative of the water body as a whole

Samples should be taken at exactly the same designated place on each sampling visit. The grab samples should be taken just below the surface of the water.

Samples should be collected directly into the sample container or in a stainless steel bucket. The mouth of a sample container should face upstream to avoid contamination [IWQS, 1997]. Containers should not be rinsed. Contamination by hands and contact with plastic or rubber should be avoided.

Containers should be filled to the brim to eliminate air, and sealed well to avoid leaking, loss of volatile chemicals and contamination. The containers should be clearly labelled (using water resistant paper and a waterproof pen), noting the sample source, sample location, sample identification, sampling depth, date and time of collection, name of sampler, etc.

4.2.4 Sample volumes

The toxicity tests required to be performed will strictly depend on the ecological category and water use category assigned to the water resource being monitored. This can result is significant savings when the resource is being managed for a category 2 (since the yeast and algae tests need not be done). Table 4.1 shows the appropriate sample volumes for various scenarios.

Table 4.1. Sample volumes (assumes all toxicity tests performed at a single laboratory).

Scenario		Toxicity tests required	Volume needed for toxicity tests (ℓ)	Volume needed for toxicants (ℓ)
Ecological category	Water use category			
1	1	Fish, *Daphnia*, Algae, Yeast	8	1.5
1	2	Fish, *Daphnia*, Algae	3.5	
2	1	Fish, *Daphnia*, Yeast	8	
2	2	Fish, *Daphnia*	3.5	

These volumes are based on the following specific requirements for each test:

Table 4.2. Sample volume requirements.

Fish	*Daphnia*	Algae	Yeast	Toxicants
1 ℓ	2.5 ℓ	50 mℓ	4.5 ℓ	1.5 ℓ

However, since formal classifications may only be carried out some years from now, in the interests of creating capacity, initially all tests should be performed on all samples. Therefore, 8 ℓ samples should be taken for the toxicity tests and 1.5 ℓ samples collected for the toxicant analyses.

4.2.5 Sample preservation

If samples to be analysed for organic toxicants are preserved with mercuric chloride then they can take up to four days to arrive at the laboratory. If they are not preserved, they must reach the laboratory within 24 hours. Preservatives must never be added to any samples to be tested for toxicity.

Therefore, whether or not a preservative should be added to samples to be analysed for toxicants depends on whether or not the samples are being sent to a single laboratory for both types of analyses:

- *One laboratory to be used for both toxicity and toxicants*. No preservatives should be added to the samples (since samples must reach the laboratory within 24 hours anyway for the toxicity tests).
- *Different laboratories to be used for toxicity and toxicants*. In this case, samples to be analysed for organic toxicants could be preserved with mercuric chloride if it is more convenient or cheaper to transport them to the organic laboratory within four days, instead of within 24 hours. However, it is preferable that no preservative is present at all during sampling to prevent accidental addition to samples intended for toxicity tests (since a preservative must not be added to such samples). Therefore, if possible, no samples should be preserved and all should reach both laboratories within 24 hours.

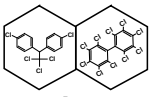
4.2.6 Sample transport

Transport samples well covered in ice (at about 4ºC, not frozen), in a dark insulated container. Samples intended for toxicity tests must be delivered to the analytical laboratory within 24 hours. If necessary use should be made of those courier companies that can guarantee that samples will be delivered within this time.

4.2.7 Sample storage in laboratory

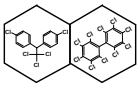
Samples must be stored in the laboratory at 4ºC in the dark (not frozen). The following table indicates how long samples may be stored before testing and extraction starts.

Table 4.3. Sample storage times in laboratory.

 **Fish**	 ***Daphnia***	 **Algae**	 **Yeast**	 **Toxicants**
1 day		Filter immediately and test within 3 days	Extract within 3 days and test within 3 days	*If not preserved*: Extract within 1 day and analyse within 1 day. *If preserved*: Extract within 7 days and analyse within 14 days

4.2.8 Summary

Table 4.4. Summary of sampling, preservation, transport and storage procedures.

	 **Fish**	 ***Daphnia***	 **Algae**	 **Yeast**	 **Toxicant analyses**	
					Recom-mended	**Alternative**
Approximate sample volume to be collected	1 ℓ	2.5 ℓ	50 mℓ	4.5 ℓ	2.5 ℓ	
Container	Clear glass Schott bottle	Clear glass Winchester bottle	Clear glass Schott bottle	Consul clear glass wide mouth jar	Dark glass Winchester bottle	
Preservative	None					1 mℓ of a 10 mg/mℓ mercuric chloride solution per bottle
Transport conditions	Keep at 4 ºC in the dark					
Transport time	To be delivered to the laboratory within 24 hours					To be delivered to the laboratory within 4 days
Storage conditions	Keep at 4 ºC in the dark. Do not freeze.					
Storage time before testing, extraction and analysis	Test within 1 day		Filter immediately and test within 3 days	Extract within 3 days and test within 3 days	Extract within 1 day and analyse within 1 day	Extract within 7 days and analyse within 14 days

4.3 ANALYTICAL PROCEDURES

4.3.1 Sample preparation for toxicity tests

Unfiltered water samples should be tested to expose test organisms to any particulate matter to which toxicants may be adsorbed. Although this is normal procedure for fish and *Daphnia* tests, this cannot be done in the algae test. In the yeast test, organic toxicants are concentrated by passing through a resin column that also contains a glass plug to remove excessive particulate matter. This cannot be avoided and may result in some loss of toxicants adsorbed to particles.

The pH, dissolved oxygen (DO), electrical conductivity and temperature of samples should be measured before use. Samples should be vigorously shaken to ensure homogeneity and to re-suspend particulate matter. The appearance of the sample (colour, turbidity, odour) should also be noted [Slabbert, 2004].

4.3.2 Infrastructure and equipment for toxicity tests

Table 4.5 provides a summary of organism maintenance for each of the toxicity tests, as well as details of special infrastructure and instrumentation required for the tests. "Routine" maintenance includes feeding, culturing/subculturing and breeding of organisms, cleaning of holding containers on a regular basis (*e.g.* fish are fed once or twice a day).

Table 4.5. Infrastructure and equipment for toxicity tests.

	 **Fish**	 ***Daphnia***	 **Algae**	 **Yeast**
Organism maintenance	Routine	Routine	Routine, refrigerate	Freeze (-70 ºC)
Special infrastructure	Temperature controlled rooms and tanks	Temperature controlled rooms	Temperature controlled rooms, illumination	Class II laminar flow, incubator, freezer
Dedicated instruments and equipment	Microscope	None	Microplate reader, microplate shaker, microscope, haemacytometer	Microplate reader, microplate shaker

4.3.3 Semi-static zebra fish development test

Recommended method for fish (*Brachydanio rerio*)

ISO, 1999.
Water quality – Determination of toxicity to embryos and larvae of freshwater fish – Semi-static method.
ISO 12890,
Geneva.

Maximum turnaround time = 14 days

4.3.3.1 Sample preparation

The samples must be stored in the dark at 4ºC for the 10 day duration of the test. Tests are carried out directly on unfiltered samples. Approximately 50 mℓ of the sample must be allowed to reach a temperature of 26±2 ºC before use.

4.3.3.2 Test method

Newly fertilised eggs (2 to 4 h old) are exposed to 25 mℓ test and control samples in Petri dishes (inner diameter: approximately 100 mm; equipped with cover) for a period of 10 days. (The standard exposure period is 10 days, but the period may be prolonged to 14 days to increase sensitivity if desired). No food is provided during the test and water is renewed daily.

Synthetic moderately hard water is used for control testing. Two Petri dishes are used for test samples and four for the control. 15 randomly selected eggs are placed in each dish (0 hours).

Eggs should not come in contact with air during transfer. For practical purposes eggs are regarded as embryos. The dishes are covered and incubated at 26±2 ºC (water temperature) under normal laboratory illumination with a 14 hours/10 hours light/dark cycle. After 24 hours the numbers of unhatched eggs in the Petri dishes are recorded and the number of viable eggs is reduced to a maximum of 10 per dish.

The determination of mean hatching times and survival is based on the 10 individuals that are now 24 hours old. At the same time every following day the viable eggs and live larvae are transferred to fresh sample in new test dishes. The number of unhatched and hatched eggs, and dead larvae, are recorded every morning and afternoon (exact time). Viable eggs are transparent while unhatched eggs are a translucent white colour.

The data are used to calculate short- (< 96 hours) and long-term (> 96 hours) lethality, as well as long-term sub-lethal toxicity.

% Embryo (egg) lethality (short-term) = $(N_{Et24} – N_{Etx})/N_{Et24}$ x 100, where:

N_{Et24} : Number of live embryos at t = 24 hours
N_{Etx} : Number of live embryos (not yet hatched) at t = 48 to 96 hours

% Larval lethality (long-term) = $(N_{Lt48\text{-}96} – N_{Ltx})/N_{Lt48\text{-}96}$ x 100, where:

$N_{Lt48\text{-}96}$: Number of live larvae (hatched) at t = 48 to 96 h
N_{Ltx} : Number of live larvae at t = 10 days

Lethality > 10 % indicates toxicity.

% Effect on hatching time (long-term sub-lethal) = $(HT_c – HT_t)/HT_c$ x 100, where:

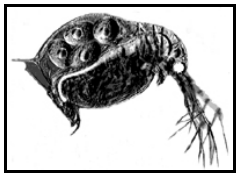
HT_c : Mean hatching time of control
HT_t : Mean hatching time of test

Effects > 20 % indicate long-term sub-lethal toxicity.

The following requirements should be met by the test to be valid:

- Control DO has been maintained at between 70 and 110 % of saturation.
- Control pH has been maintained at 7.5 ± 0.2.
- Test temperature has been maintained at 26 ± 2ºC.
- Control embryo lethality (initial 15 eggs) after 24 hours should be < 30 %.
- Control embryo lethality (final 10 eggs) after 48 to 96 hours should be ≤ 10 %.
- Control larval lethality after 10 days should be ≤ 10 %.
- Control mean hatching time should be between 2 and 4 days.

4.3.4 *Daphnia pulex* reproduction test



Recommended method for invertebrates (*Daphnia pulex*)

Slabbert JL., 2004.
Methods for direct estimation of ecological effect potential (DEEEP).
WRC Report No. K5/1313/1/04,
Pretoria.

Maximum turnaround time = 23 days

4.3.4.1 Sample preparation

The samples must be stored in the dark at 4 ºC for the 21-day duration of the test. Tests are carried out directly on unfiltered samples. Approximately 50 mℓ of the sample must be allowed to reach a temperature of 26 ± 2 ºC before use. Pathogenic and/or predatory organisms in samples may affect survival. Remove interfering organisms by means of a pipette or if there are too many filter the sample through Whatman No. 1 filter paper.

4.3.4.2 Test method

Daphnia, less than 24 h old, are exposed to test and control samples for a period of 21 days in a semi-static test. 10 *Daphnia* are used per sample. Each organism is placed in a separate 50 mℓ beaker containing 25 mℓ sample. Moderately hard water is used for control testing.

The survival of the parents, the presence of males and ephippia, and the number of live offspring produced per live parent is recorded every second day when test solutions are renewed and *Daphnia* are fed. At this time the parent animals are transferred to the fresh sample in a second series of beakers by means of a pipette of suitable bore diameter. Before distributing samples into test beakers, add 375 µℓ TYA (trout pellet, yeast and alfalfa) food and 2 mℓ algal suspension to 250 mℓ sample and mix well (algal concentration in test solution: 2 x 10^5 cells/mℓ).

Young *Daphnia* are very susceptible to air entrapment, and should be handled with a pipette rather than being poured. Beakers are covered with plastic or glass plates to limit evaporation. The test is carried out in a temperature controlled facility at 20 ± 2 ºC under normal laboratory illumination (artificial and/or daylight: 10 to 14 hours light). Variations in ambient light intensities and prevailing day/night cycles in most laboratories do not seem to affect *Daphnia* growth and reproduction significantly.

The data are used to calculate short- (< 96 hours) and long-term (> 96 hours) lethality, as well as long-term sub-lethal toxicity.

% Short-term lethality = $(N_{t0} - N_{tx})/N_{t0} \times 100$, where:

N_{t0} : Number of live *Daphnia* at t = 0 hours
N_{tx} : Number of live *Daphnia* at t = 96 hours

% Long-term lethality = $(N_{t0} - N_{tx})/N_{t0} \times 100$, where:

N_{t0} : Number of live *Daphnia* at t = 0 days
N_{tx} : Number of live *Daphnia* at t = 21 days

Lethality >20 % indicates toxicity.

% Inhibition in reproduction = $(N_c - N_t)/N_c \times 100$, where:

N_c : Mean number of offspring per live control parent at t = 21 days
N_t : Mean number of offspring per live test parent at t = 21 days

Inhibition > 20 % indicates toxicity.

The following requirements should be met by the test to be valid:

- Control DO has been maintained at > 40 % of saturation.
- Control pH has been maintained at between 6 and 8.5.
- Test temperature has been maintained at 20 ± 2 ºC.
- *Daphnia* have been exposed for at least 21 days.
- Adult lethality in the control is < 20 % after 96 hours.
- Adult lethality in the control is < 20 % at the end of the test.
- Living males in the control are ≤ 20 % at the end of the test.
- The mean number of offspring per parent in the controls is > 40.

4.3.5 Algal 24-well microplate growth inhibition test

Recommended method for algae (*Selenastrum capricornutum)*

Slabbert JL., 2004.
Methods for direct estimation of ecological effect potential (DEEEP).
WRC Report No. K5/1313/1/04,
Pretoria.

Maximum turnaround time = 5 days

4.3.5.1 Sample preparation

As soon as possible after sample receipt, approximately 50 mℓ of the water is filter sterilised using a syringe and a sterile 0.22 µm syringe filter. The sample is kept in a sterile 100 mℓ Schott bottle at 4ºC until use (usually within 3 d). The sample should be allowed to reach a temperature of 20 ± 2 ºC before use.

4.3.5.2 Test method

Logarithmically growing cells of *S. capricornutum* Printz are exposed to test and control samples over several generations for a period of 72 hours, in a static 24-well microplate system, using defined conditions. 200 000 cells/mℓ (3 to 4 days old logarithmic phase algal culture) are used for sample inoculation. The algal suspension is added at a ratio of 1:1 to a 20-times concentrate of the culture medium and used as 200 µℓ volumes for inoculation of 1.8 mℓ sample in test wells (well volume: 3.5 mℓ). The top row of each plate is used for blanking and receives sample and medium only (no algal cells).

Plates are read with a microplate reader (450 nm) to determine the optical density (OD) at t=0. Incubation is carried out at 24 ± 2 ºC using continuous illumination (cool white fluorescent light - 80 to 95 µE/m^2/s). At the end of the incubation period cells are suspended using a microplate shaker, and growth measured in terms of OD. If the growth in the controls does not meet the stated OD validity of 0.150 ± 0.03 after 72 hours incubation, plates can be incubated for a further period, but not longer than 96 hours.

The data are used to determine long-term sub-lethal toxicity.

$$\% \text{ Growth inhibition} = [(ODC - OD_0) - (ODT - OD_0 - ODB_{>0.005})]/(ODC - OD_0) \times 100,$$

where

OD_0:	Mean OD of six control wells at t = 0 hours
ODC:	Mean OD of six control wells on each plate at t = 72 hours
ODT:	Mean OD of three test wells on each plate at t = 72 hours
$ODB_{>0.005}$:	Blank OD in the corresponding test wells >0.005 (to compensate for colour and/or precipitation)

Inhibition ≥ 20 % indicates toxicity.

Algal growth can also be stimulated. A stimulation ≥ 20 % usually indicates excess nutrients. If stimulation is observed, this should be recorded.

The following requirements should be met by the test to be valid:

- The coefficient of variation in the control should be < 10 %.
- Control OD after 72 hours should be 0.150 ± 0.03.

4.3.6 Recombinant yeast (hER) test (humans)

Recommended method for humans (recombinant yeast test)

Slabbert JL and EA Venter, 2005.
Method development for biochemical procedures related to estrogen and androgen screening of water and sediment samples.
WRC Draft Report No. K8/478,
WRC EDC Programme K5/1402,
Pretoria.

Maximum turnaround time = 14 days

4.3.6.1 Sample preparation

Testing water for hormones or estrogen mimics consists of an initial stage of passing a large volume of water through a column of adsorbing medium in order to concentrate any hormones/estrogen mimics that may be present. The larger the sample volume, the more sensitive the test will be.

Concentrate the sample as soon as possible after receipt. Pass 4 ℓ of the sample through Amberlite XAD-7 resin packed in glass columns [Slabbert, 2004]. XAD-7 is a moderately polar acrylic resin that can adsorb a wide range of organic substances. Recover the adsorbed organic substances by elution (desorption) using acetone. Concentrate the eluant by rotary evaporation and then dissolved in 1.0 mℓ reagent grade ethanol (4 000 times concentration). Store the extracts in sterile Vacutainers at -20 ºC. Before testing, place the samples in finely ground ice in an ice box. Use the cold samples to prepare the required dilution series for the test.

4.3.6.2 Test method

The test is based on the metabolism of yellow chlorophenol red-ß-D-galactopyranosid (CPRG) to a red product, which can be assessed spectrophotometrically. The colour reaction is due to the action of ß-galactosidase, which is encoded by the reporter gene *lac-z,* carried by expression plasmids in a transfected yeast cell. The human oestrogen receptor (hER) is also inserted into the yeast cell. Binding of a hormone/chemical compound to the receptor thus induces expression of the *lac-z* gene, which leads to the activation of ß-galactosidase.

Logarithmically growing yeast cells (*Saccharomyces cerevisiae*) are exposed to serial dilutions of ethanol extracts of water samples in 96-well microplates for 10 days, using defined conditions. Each microplate also contains a positive (17ß-estradiol) and negative control (100 % ethanol). The test is carried out under sterile conditions in duplicate flat-bottomed 96-well microplates [Routledge and Sumpter, 1996; Slabbert *et al.,* 2005].

Samples, as well as the positive control, are serially diluted in 100 % ethanol. 10 µℓ of the test sample and controls are placed in the microplate wells. An overnight culture is diluted with fresh growth medium to an optical density of approximately 1.0. Exactly 0.4 mℓ of this preparation and 0.5 mℓ CPRG, are added to 50 mℓ growth medium (assay medium). After

evaporation of the ethanol from microplate wells, 200 µℓ of the assay medium are introduced into each well. Plates are sealed with Parafilm, shaken on a microplate shaker for 2 min, and incubated at 32 ± 1 °C for 3 days, followed by a further incubation period of 7 days at room temperature. Plates are shaken for 5 min and allowed to stand for 1 hour before absorbance measurements. Absorbance is measured with a microplate reader at 550 and 600 nm, to account for the CPRG colour change and turbidity (yeast growth), respectively.

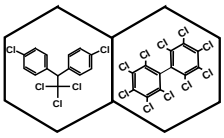
Absorbance values are imported into a calculation template in Microsoft Excel. To correct for turbidity (each well), the following equation is applied to the data:

$$\text{Corrected absorbance} = Abs_{550\ nm} - [Abs_{600\ nm} - Abs_{\text{negative control, 600 nm}}]$$

The final corrected absorbance values represent the data from two plates. The data are used to determine long-term sub-lethal toxicity (estrogenicity and growth inhibition). The positive control concentration and absorbance values and test absorbance values are the data for non-linear regression (sigmoidal dose-response). Matching concentrations (nanogram estradiol equivalents) for test absorbance values are obtained and EC_{50}s (concentrations causing 50 % induction) calculated. EC_{50} values are used to calculate the Relative Potency (RP) (Estradiol EC_{50}/Water EC_{50}) and the Relative Induction Efficiency (RIE) (Max $Absorbance_{water}$/Max $Absorbance_{estradiol}$).

When the calculation of EC values is not possible (too few values above the blank), the results are expressed as either positive or negative. Three absorbance values above the mean negative control+3SD (standard deviation) indicate significant induction, while three values below the mean negative control-3SD is an indication of significant cytotoxicity.

4.3.7 Toxicants



Recommended method for Persistent Organic Pollutants (POPs)

AOAC Official Methods of Analysis, 1995.
Pesticides and Industrial Chemical Residues.
Organochlorine Pesticides in Water, Gas Chromatographic Method,
Method 990.06,
16th Ed. Vol 1, Chapter 10, 13-17. Association of Official Agricultural Chemists.

Maximum turnaround time = 2 days (10 samples)

4.3.7.1 Infrastructure and equipment

A temperature controlled room is required. A rotavapour concentrator and a Gas Chromatograph – Mass Spectrometer (GC-MS) is required for the recommended method. However, other detectors such as electron capture, nitrogen phosphorous and a flame photometer can also be used.

4.3.7.2 Sample preparation (extraction)

1 ℓ of water (no pH adjustment and unfiltered) is extracted with the organic solvent dichloromethane (DCM), which has a low volatility and is suitable for extraction of a wide range of organic compounds. DCM is added to the sample in a separatory funnel. Following standard procedures, the funnel is shaken vigorously by hand, with periodic venting to release excess pressure. After separation of the liquid phases, the extract is dried with anhydrous sodium sulphate. The extract is then concentrated to 5 mℓ with a rotavapour and stored in PTFE Teflon sealed screw-cap vials at 4ºC. If a higher concentration is required the sample could be concentrated to a smaller volume, *e.g.* 1 mℓ. Since the listed toxicants are very stabile in organic solvents at low temperatures the extract can be stored for a period of up to one month.

4.3.7.3 Analytical method

A Gas Chromatograph – Mass Spectrometer (GC-MS) is used to quantify and confirm the organic toxicants. Standard GC operating and maintenance procedures are followed. The original method should be consulted for specific operating conditions and retention times. The calibration curve is verified daily using one or two calibration standards according to RQS standard operating procedures.

The minimum quality control (QC) requirements are as follows:

- An initial demonstration of the method performance.
- The analysis of a surrogate standard in each sample (acceptable recovery: 70 to 130 %).
- The monitoring of internal area counts in each sample (should be within 30 % of area in calibration standard).
- Analysis of method blanks as continuing check on sample contamination.

- The analysis of spiked samples as a continuing check on method recovery.
- The analysis of the instrument QC standard to ensure acceptable instrument performance.

The detection limit for the PCB congeners is 10 ng/ℓ. The organochlorine pesticides are detected at between 10 and 100 ng/ℓ, monochrotophos at between 100 and 500 ng/ℓ and the triazines at 1 000 ng/ℓ. See Section 5.3.5 for how to deal with situations where the detection limit is higher than the associated guideline value.

In the event of a laboratory not having access to a mass spectrometer, the following alternative detectors can be used:

- Electron capture for the PCBs and organochlorine pesticides. For this detector methyl tertiary-butyl ether (MTBE) is the preferred organic solvent.
- Nitrogen Phosphorous for triazines.
- Flame Photometer for the organophosphate monochrotophos.

However, it should be kept in mind that these systems are only used for quantification. A column with a different phase will be required for confirmatory purposes.

CHAPTER 5: DATA MANAGEMENT & REPORTING ("DATA & INFORMATION")

This chapter describes how the monitoring data should be managed and how it should be assessed and reported.

5.1 INTRODUCTION

With samples taken and analyses and tests performed, management of the results and their appropriate presentation are the final critical steps needed to achieve the programme's objectives.

This chapter describes specifically how the monitoring data should be managed and how they should be assessed and reported. The NTMP will rely heavily on existing protocols being practised at the Directorate: Resource Quality Services (D:RQS) in respect of the Water Management System (WMS). Most of these are quite adequate for the NTMP and are therefore not reproduced here. This chapter rather focuses on those aspects that are NTMP-specific and that are not yet common practice.

5.2 DATA MANAGEMENT

5.2.1 Introduction

Data management involves a wide range of activities from properly registering the monitoring programme (monitoring points, monitoring frequency, etc.), managing sampling (printing schedules for monitors and laboratories, printing sample tags, etc.), receipt of analytical results (measured either in a laboratory or on-site), to capturing these results on WMS and making them available for subsequent processing (*e.g.* reporting).

WMS, situated at D:RQS near Roodeplaat Dam north of Pretoria, will be the central database used for all data associated with the NTMP.

5.2.2 Registration

Registration of the programme and individual monitoring points on WMS is done using standard application forms that can be found on the WMS website:

http://www-dwaf.pwv.gov.za/iwqs/wms/indexforms.htm

Users with direct access to WMS can perform their own registration or it can be done by D:RQS.

The following information can be used to facilitate registration of the NTMP:

- **Monitoring Programme Name:** National Toxicity Monitoring Programme
- **Monitoring Programme Description:** Monitoring the status and trends of toxic effects to selected organisms, and selected potentially toxic substances, to support strategic management decisions in respect of fitness for domestic use and aquatic ecosystems.
- **Programme Priority Number:** 90 (number of days between sampling and results being available)
- **Transport method:** Direct delivery.
- **Packaging method:** Cooler bag or box.

5.2.3 Sample management

Sample management is an important function related to data management. This involves managing the links between details of the monitoring programme, the individual monitoring points, the sampling procedure and ultimately the analytical data. It specifically comprises the following actions:

For the sampler/monitor the following must be produced:

- A monitoring schedule for each monitoring point. This describes in detail where, when and how samples should be taken.
- Sample tags. These accompany sample bottles to laboratories and contain a variety of data that identify where, when, how and by whom samples were taken.

For each participating laboratory the following must be produced:

- An analysis schedule describing time frames, types of samples, monitoring variables to be analysed and what analytical methods should be used.

5.2.4 Data capture

5.2.4.1 From D:RQS laboratory

If the D:RQS laboratory performs the analyses, the standard procedures currently in place should be used to capture the data on WMS. These should include all the usual quality control checks for mistakes and data values that lie outside expected ranges.

5.2.4.2 From remote laboratory

The Department continuously strives to develop and implement efficient methods to facilitate direct data capture from remote laboratories onto WMS. However, until efficient direct capture is achieved, remote participating laboratories should use a standard Excel spreadsheet template to capture their data locally and to transmit these data via email to D:RQS for automatic capture on WMS. The latter can be achieved by developing specific protocols tailor-made to the format of the spreadsheet. This spreadsheet should have the following properties:

- It must be simple to use ("user-friendly").
- It must be relatively small in size (preferably much less than 1MB) to facilitate Emailing as an attachment.
- It should apply a general philosophy that is as consistent as possible with current and likely future thinking associated with WMS.
- It must check all entered data against lower and upper absolute and practical limits (see Table 5.1 below).
- It must be in a format that facilitates efficient importing of the data into WMS.
- It must be in a format that facilitates efficient importing of the data into the NTMP data assessment spreadsheet (described in Section 5.3 below).

The purposes of this procedure are as follows:

- To minimise human error by minimising the number of times data are entered manually.

- To ensure that the simple basic checks for mistakes or unusual values take place at the laboratory (not after transmission to D:RQS). This avoids delays when problems are detected.
- To ensure data are captured as efficiently as possible onto WMS.

The following table shows the limits against which captured values can be checked for mistakes.

Table 5.1. Lower and upper limits (absolute and practical) for checking individual data entries.

Variable	Units	Absolute lower limit	Absolute upper limit	Initial practical lower limit	Initial practical upper limit
Any toxicity reported as a percentage	%	0	100	Detection limit	100
Yeast test (pos./neg.)		0	1	0	1
Yeast test	g eq estradiol	0	Not defined	10^{-10}	1 000 000
Any organic toxicant (POPs, etc.)	µg/ℓ	0	Not defined	Detection limit	10 000

The *absolute limits* are those values within which all data must theoretically occur (*e.g.* one cannot have a percentage greater than 100 or less than zero). *Practical limits* are defined here as those values beyond which a measurement is very unlikely to occur. However, it is not necessarily impossible for this to happen. Lower practical limits are usually taken to be detection limits. The following table suggests actions when a datum is not within the designated limits.

Table 5.2. Actions when a datum is not within limits.

Observation	Action
Datum < absolute lower limit, or Datum > absolute upper limit	Reject datum. Advise data capturer that datum must be within, or equal to, the defined absolute limits.
Datum < practical lower limit	Advise data capturer that practical lower limit is defined as the detection limit and that entered value is less than this limit. If detection limit has changed, then recommend the practical lower limit be changed to equal the new detection limit. Otherwise, insist that entered value corresponds to "less than detection limit" (or ND for "Not detected").
Datum > practical upper limit	Advise data capturer (a) that the entered value is very high and (b) to check that value is correct. If no obvious problem is immediately apparent, advise re-analysis. If re-analysis confirms high value, accept high value.

5.2.5 Data retrieval

The nature of the data retrieval from WMS will depend on the purpose to which the data will put. However, data are usually exported from WMS in delimited ASCII format. This facilitates simple importing into a variety of other software packages. The following are some examples.

5.2.5.1 Annual reporting

The data assessment spreadsheet (described below) can be used to assess the data and prepare written annual reports. To facilitate this, all the necessary data must be retrieved from WMS and imported into the spreadsheet. Data for single monitoring points should be retrieved for the current and previous year. The ASCII file should be opened in Excel and the individual cells (of the raw data) in the assessment spreadsheet linked directly to this.

5.2.5.2 Quality control

Regular examination of results to date should be carried out to monitor the progress of the programme, particularly from the point of view of missing data and general adherence to programme design. The following are a few examples of what data can be retrieved for these purposes:

- An inventory of all the monitoring stations and monitoring variables analysed in a specific drainage region (primary, secondary, tertiary, or quaternary.), from which specific monitoring points can be selected.
- Water quality sampling results for specific variables.
- Summary statistics of the sampling results.
- Information available for specified monitoring points (for a specified period).

5.3 INFORMATION GENERATION AND DISSEMINATION

5.3.1 Introduction

An important aspect of information generation is ensuring that misinterpretation of the data and the assessment is avoided. This is particularly so in the field of toxicity. "Toxicity" could be perceived by some to be an emotive word conjuring up all manner of undesirable images of a water resource in a dire state. This is particularly so given that the resource classification system, by its very nature, allows for selected resources to be become (and remain) degraded to some degree, though not unsustainably so, when this is perceived to be outweighed by desirable socio-economic development based on use of the water resource.

The following are a few issues that underlie the approach to assessment and reporting:

- General members of the public are not the primary target audience. This does not mean they will not have access to NTMP reports. It simply implies that some degree of competence is required in the target reader.
- Toxicity tests on aquatic organisms, besides being used to indicate potential risks to the aquatic environment are also used as a precautionary measure to indicate that there may be some risk to humans if such toxicity exists or persists.
- Although algal growth stimulation will also be recorded (if it is observed while measuring growth inhibition), it will not be reported since it does not directly relate to "toxicity". However, the data should be available to anyone who wishes to assess it.
- In general, NTMP reports should not attempt to recommend detailed management responses to potential toxicity problems that may be identified.
- Assessment and reporting should be kept simple.

5.3.2 Target users

It is important that the ultimate target users (clients) of the information provided in reports produced by the NTMP are (a) clearly identified and (b) kept in mind at all times. The following are the primary and secondary target users.

Primary users:

- The Minister of Water Affairs and Forestry
- DWAF Director General
- Water Resource Quality Managers and Water Quality Managers (DWAF head office and regional offices)
- Water Management Institutions (like catchment management agencies)
- Water User Associations

Secondary users:

- National, provincial and local government authorities
- Non Government Organisations
- All industrial sectors
- Public
- Any other interested party

5.3.3 Data assessment spreadsheet

A spreadsheet exists that facilitates plotting of raw data and performing a series of data assessments. This has been formatted in such a way that worksheets can be conveniently printed on A4 pages. This can be used to produce a written report. It is also used to produce the data required for spatial presentation (maps) of monitoring results.

The spreadsheet itself can also be supplied to water quality managers who can perform various operations themselves if they so require.

5.3.4 False positive and false negative results

The null hypotheses given in Chapter 3: Monitoring Framework provide a framework for understanding the causes and consequences of two kinds of errors that can be made. These are most usefully referred to as "false negative" and "false positive" results. The word "positive" refers to reporting that the null hypothesis (whatever it might be) is true.

- A "false negative" refers to reporting a negative result (*i.e.* that the null hypothesis is false) when it should have been reported as positive.
- A "false positive" result refers to incorrectly reporting a positive result (*i.e.* the result was actually negative).

Table 5.3 shows some of the possible causes of these errors. It is assumed that the null hypothesis is simply "there is a toxicity problem".

Table 5.3. Possible causes of false negative and false positive results.

	Causes of false negative results	Causes of false positive results
Sampling method	Snapshot sampling that may miss toxicant peaks or spikes.	
Monitoring point		Sampling in an effluent plume or mixing zone (collecting a sample that is not representative of the catchment).
Sensitivity	Insensitive tests.	Overly sensitive tests.
Bias	Using toxic criteria that are too lenient.	Using toxic criteria that are too strict.
Variability	Highly variable toxicity test results in the vicinity of the toxic criterion can report no problem when there is actually a problem (see Figure 5.1).	Highly variable toxicity results in the vicinity of the toxic criterion can report a problem when there is actually no problem (see Figure 5.1).

The following figure illustrates how variability in a measurement causes false negative and false positive results. (Hypothetical values have been chosen although such a high variability is possible with certain toxicity tests. The "true value" is also not always in the centre of the range.)

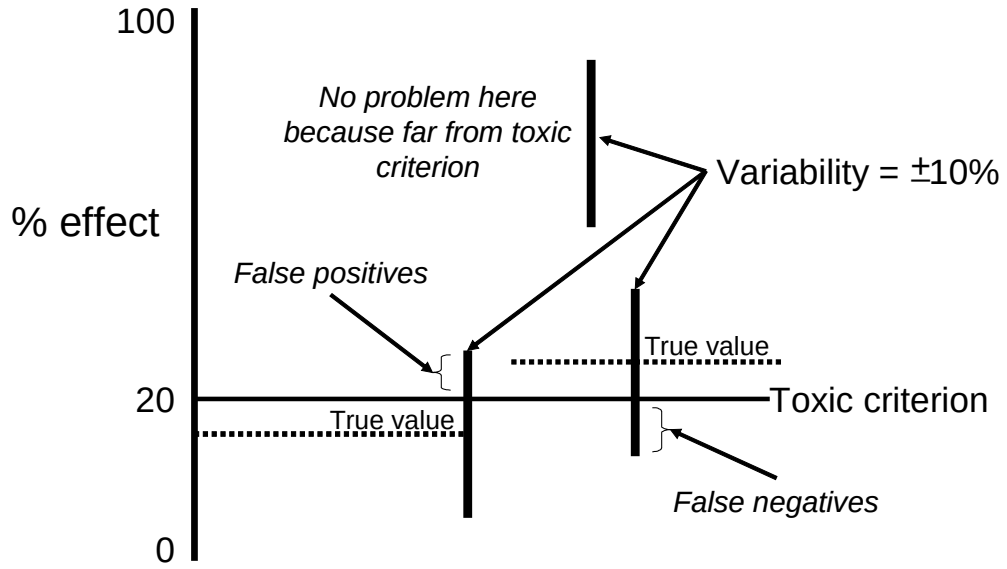


Figure 5.1. Illustration of how variability can cause false negative and false positive results.

Table 5.4 summarises some of the consequences of these errors. These issues need to be borne in mind when interpreting and acting on (or not acting on, as the case may be) any monitoring results.

Table 5.4. Summary of consequences of false negative and false positive errors.

	False Negatives	False Positives
Ecosystem integrity	Inadequate protection of water resources	Over-protection of water resources and decreased cost-effectiveness of NTMP
Fitness for use	Increased likelihood of negative impacts on water users (and socio-economic enhancement and optimal water use)	

5.3.5 Guidelines

5.3.5.1 Toxicity

As noted in Chapter 3: Monitoring Framework, Section 3.3, the criteria against which toxicity measurements should be compared to test the null hypotheses are those in Table 3.2. In summary, for category 1 the water must not show any toxicity of any kind. For category 2, the water must not show any lethality (short- or long-term), although some sub-lethality may be observed. Although the toxicities are measured quantitatively (typically as a percentage effect) and recorded as such, for purposes of testing the null hypotheses in Chapter 3 no distinction is made between different levels of lethality or sub-lethality. For example, 20 % fish lethality would place that water resource in a category 3, just as 100 % lethality would. Similarly, 30 % *Daphnia* sub-lethality (and no lethality) would place the resource in category 2, just as 100 % sub-lethality (and no lethality) would.

5.3.5.2 Toxicants

Protocols have been developed for determining guidelines for toxicants for both domestic use and aquatic ecosystem protection. The following reports can be consulted for the details:

Domestic use: Genthe and Steyn, 2005.

Aquatic ecosystems: Warne *et al.*, 2005.

The latest guidelines determined using these methods should be consulted for the numerical values.

It is conceivable that a guideline may demand an extremely low detection limit that cannot easily be achieved with routine analytical procedures. Although it may be technically possible to achieve this lower detection limit using procedures that are not routine, the extra resources required to do so may increase the analytical costs to a level that makes it difficult to justify. A number of scenarios could then be investigated, including the following:

Scenario 1: Accepting high detection limits

One scenario is to analyse for the toxicant to a financially feasible detection limit above the guideline and accept the associated lower information content. Figure 5.2 illustrates the various circumstances when this is a problem using the ecological category as an example.

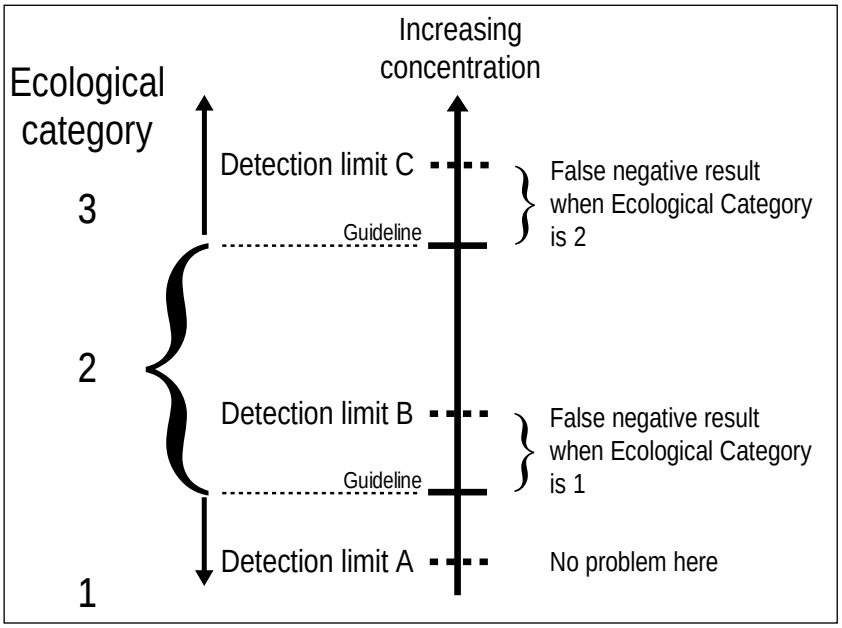


Figure 5.2. Illustration of when high detection limits will be a problem.

If the detection limit (A) is less than the lowest guideline value then there is no problem.

If the ecological category has been designated 1 and the detection limit (B) is higher than the category 1 guideline, a false negative result is only obtained when the real concentration is between the guideline value and detection limit B. Real concentrations above the detection limit will, by definition, be detected and there is no problem. Real concentrations below the category 1 guideline will also not be detected but this is also strictly not a problem.

The same logic applies when the ecological category is 2 and detection limit C is the best the analytical method can do. However, if the ecological category is 1 this is totally unacceptable since the detection limit is so high that concentrations even in category 3 would not be detected. Table 5.5 summarises these and other possible scenarios.

Table 5.5. Summary of when detection limits will be a problem.

	Detection limit less than category 1 guideline	**Detection limit between category 1 guideline & category 3 guideline**	**Detection limit greater than category 3guideline**
Ecological category = 2	No problem	No problem	Problem when real concentration between category 3 guideline and detection limit
Ecological category = 1	No problem	Problem when real concentration between category 1 guideline and detection limit	Totally unacceptable

In the interim before water resources are classified, the equivalent of the category 1 guideline will be the only guideline used. In this case only the last row of the table is relevant although the last column is not relevant.

The nature of the problem is such that it is difficult to give general quantitative guidance on when a detection limit that is greater than a guideline value might be acceptable. Qualitatively, the closer the detection limit is to the guideline value the less the problem is (*i.e.* the less the chance of a false negative result). As indicated in Table 5.7, if the ecological category is category 1 and the detection limit is greater than the category 3 guideline, this is totally unacceptable. Measures will have to be taken to lower the detection limit.

Each toxicant will need to be considered on its own merits.

Scenario 2: Drop toxicant from list

If it is very difficult or expensive to achieve lower detection limits, it may be necessary to drop the toxicant from the list entirely until new analytical technology is available that allows a sufficiently low detection limit to be achieved cost-effectively.

Scenario 3: Expand suite of toxicity tests

If it is very difficult or expensive to achieve lower detection limits, and the toxicant is dropped from the list entirely, depending on the nature of the toxicant it may be acceptable to place more reliance on the toxicity tests. If the suite of toxicity tests currently being used is not able to detect the presence of the dropped toxicant, it may be possible to include another toxicity test that can.

5.3.6 Avoiding misinterpretation in written reports

5.3.6.1 Being "soundly scientific"

The objectives of the NTMP state that the monitoring should be "soundly scientific". It is very important that assessments of data are both accurate and absolutely defensible. As an incentive for it being objective and scientific, it is useful to imagine that the assessment might need to be defended in court. Part of being objective is ensuring that assessments support decision making that is genuinely *informed*.

Assessment of monitoring results can largely be based on the facilities provided by the assessment spreadsheet. However, they must be accompanied by appropriate text that ensures that misinterpretation of results is avoided. Some examples are given in the following sub-sections.

5.3.6.2 Detection limits higher than guidelines

If the decision is taken to accept a detection limit higher than a guideline value, then it is imperative that the following be noted in reports when measurements are below detection limits.

"*Values below the detection limit for this toxicant may be misleading because the detection limit is higher than the guideline value. In other words, it cannot be confirmed that the water resource is in fact the designated category.*"

5.3.6.3 Bias of national coverage

The very nature of priority area monitoring, which focuses specifically on areas suspected or known to experience toxicity-related problems, will mean that presentation of results (whether tabular or spatial) will be biased. It is therefore important that appropriate text

appears in any such report that makes the reader aware of (a) this bias and (b) that it is intentional. For example, the following could be used:

"*The location of monitoring points has intentionally been focused in areas known, or suspected, to experience toxicity-related impacts. For example, they have been chosen in areas where:*

- *Land use is such that selected toxicants can potentially enter surface water resources, and*
- *Water is, or may be, used for domestic purposes (either directly or after treatment), or*
- *Sensitive and / or important aquatic ecosystems exist.*

Many areas that are not currently suspected of experiencing toxicity-related impacts are not yet being monitored. Therefore it is not possible to draw firm conclusions about the area reported on as a whole. However, it is possible that the results of this report present an impression of a status for the area as a whole that is worse than is actually the case."

5.3.6.4 Choice of monitoring points

The reasons why each monitoring point was chosen for the NTMP should appear in the report. This can appear in a table in an appendix or in the body of the report. These are important for providing a connection between the individual point being monitored and the area it is intended to represent. They provide a more specific (and local) context in which the interpretation of results by the reader can take place. Examples of such reasons are the following:

- Catchment has significant agricultural land use and sensitive wetlands in the <name> Ramsar site.
- Pesticide manufacturing plant exists at <place> and water abstracted downstream by the <name> water treatment works for bulk water supply to <town>.
- DDT used in upstream catchment for malaria control and water used downstream in neighbouring Mozambique for domestic use at <place>.

5.3.6.5 Presence or absence of toxicants

Whether or not a measured toxicant concentration exceeds its guideline value, simply the presence of a toxicant that is known not to occur naturally in ecosystems should present some cause for concern. This may not necessarily "trigger" any specific management response (unless a guideline value is exceeded). However, simply reporting that the compound does not occur naturally provides the reader with a small piece of information. This facilitates informed decision-making by water resource managers. Therefore, if the toxicant is not a compound that occurs naturally in ecosystems, the report should say:

"*This compound does not occur naturally. Its presence therefore indicates some contamination of the water resource.*"

5.3.6.6 Interpreting trigger values

A management decision to actually respond to this contamination in some way needs to rely on a comparison of the measured values with guideline concentrations that trigger such a response. The "Trigger Values" can, for example, be expressed as a "PC95 50 %" [Warne *et al.*, 2005].

In order to ensure that the guideline values are not misinterpreted, the following text should appear in any report based on such guidelines.

A single "PC95 50 %" is the "protective concentration" (PC) that has a 50 % certainty of protecting 95 % of the species in the water resource. An alternative interpretation is that 50 % of all the PC95 50 % values will protect at least 95 % of the species.

"PC95 50 %" concentrations can be regarded as "trigger values". That is, when measured concentrations in a water resource exceed these values, certain management actions can be "triggered". (These actions may include initialising a more detailed investigation, issuing warnings to water users, remediation, etc.) They should not be interpreted literally in terms of actual species protected. In other words, simply regard these as "operational" trigger values that prompt certain actions.

These "PC95 50 %" concentrations are theoretical values determined independently of one another. They are also generic. That is, they are not specific to individual water resources. Therefore, any one specific water resource may not be protected to the theoretical degree indicated, for the following reasons:

- *Only 50 % of the trigger values will protect 9 5% of the species. The actual percentage of species protected may therefore be higher or lower.*
- *The sensitivities of the remainder of the species to the toxicant(s) may not be the same as that used to derive the trigger values.*
- *The assumptions of the statistical model used to determine the trigger values may not be appropriate.*
- *The overall toxicity in the water resource may differ from that in the laboratory due to various chemical and physical differences (like pH, level of organic matter, suspended matter, etc.).*
- *There may be uncharacterised interactions (synergism and/or antagonism) between the detected toxicants that increase or decrease the actual potential toxicity. The trigger values assume individual toxicants act independently. That is, mixtures are strictly not taken into account.*

From the point of view of the water quality manager, it is safest to regard the guideline values as simply indicators of whether or not the water resource is in the desired state (ecological category or water use category). No attempts should ever be made to interpret the guidelines, nor communicate them, in terms of the actual number of species likely to be protected.

5.3.6.7 Comparing toxicity and toxicant results

The approach used in the NTMP to establish whether the resource is in the desired category or not differs for toxicity and toxicants in a fundamental way. The toxicity results are based on the presence or absence of either "no toxicity" (sub-lethality or lethality, short- or long-term) or "no lethality" (short- or long-term) to a few selected (hopefully reasonably representative) species of three trophic levels. The toxicant guidelines for aquatic ecosystems are based more on exposure time and lethality only. On the other hand, guidelines for human health (domestic use) ensure that people are safe for a lifetime of exposure to a specific dose.

In order to prevent potentially fruitless (and inherently fundamentally flawed) rationalisations, the report must include a statement to the following effect:

"*Because the approaches to determining (a) the degree of toxicity and (b) guidelines for toxicants differ in a fundamental way, no attempt should be made under any circumstances to rationalise toxicity results with measured toxicant concentrations. The results should be accepted at face value and acted upon accordingly.*"

Maps should include the abbreviated statement: "*Note: Some apparent discrepancies are inevitable*".

In effect, this acknowledges the possibility that results may appear to be inconsistent.

5.3.6.8 Measurements below detection limits

Analytical measurements of toxicant concentrations have detection limits. Similarly, toxicity tests only report a positive result (*i.e.* observed toxicity) when the percentage effect is above a certain level (usually 20 %). This, in effect, is a kind of detection limit.

Although it is common practice to use half the detection limit as a value in statistical calculations when a measurement is below the detection limit, such values will not be displayed in graphs. Zero will rather be displayed because an uninformed reader could literally interpret a series of half detection limit values as meaning, say, continuous levels of toxicity at 10 % (assuming a detection limit of 20 %).

5.3.6.9 Confidence

For a comparison of each individual measurement against its guideline value, the assessment spreadsheet reports the maximum probability (at 99 % confidence) that what is being reported (*i.e.* the assessment against the guideline) is incorrect. For example, if the answer to the question "Is it within the guideline value?" is "Yes", this is the maximum probability that it should actually have been reported as a "No". It is important to note that this is an estimate of the maximum probability, not the most likely probability. That is, the most likely probability will usually be less than the maximum value.

Typically, non-zero values of this probability will only appear when the measured value is relatively close to the guideline value (relative to the coefficient of variation of the measurement). The purpose of this probability is to improve the ability of managers to make informed decisions by noting the degree of confidence they can have in the assessment.

The same maximum probability of being incorrect should also be calculated for "aggregated statistics" (*i.e.* based on many such measurements), such as whether the water resource is within the chosen ecological category.

Missing data also decreases the confidence the manager can have in the results. Accordingly, the percentage of missing results, as calculated by the assessment spreadsheet, should also be reported.

5.3.7 Verbal presentation

Results should be verbally presented to users whenever this is possible. This will greatly increase the chance that critical results will actually be internalised by the intended audience (who may have difficulty in finding the necessary time to read reports carefully). It will also provide a useful opportunity for that audience to provide feedback on the usefulness of the data and assessment. This information can be the basis of future reviews of the NTMP.

5.3.8 Spatial presentation

Maps should be produced that display either a "Yes" (in green) or a "No" (in red) that answer a series of questions.

First, it is necessary to provide results from the NTMP that are consistent with those currently reported in other national monitoring programmes (that are not aligned with the classification system). In effect, the question to be answered therefore needs to be the following:

- Water resource in an ecological category 1 and a domestic use category 1?

It should be noted that the above question is asked irrespective of what ecological category and water use category may have been designated. It is important to note that in the NTMP assessment spreadsheet in the "Raw Data" worksheet, the answers to the questions "Ecological category 1 (Yes/No)?" and "Water Use Category 1? (Yes/No)" must both be set to "Yes". The data are then obtained directly from the worksheet "One Point One Year Summary" for each monitoring point. Figures 5.3 to 5.5 illustrate a possible spatial format.

Although these three maps present results that are more consistent with those of other national monitoring programmes, they will ultimately be somewhat misleading. This is because the designated management class will define the ecological category and water use category for which the water resource should be managed. These will not always be category 1. Some may be category 2. These are, by definition, less demanding management objectives (than category 1). The above maps will therefore show an apparently worse overall scenario that is necessary. Accordingly, the maps illustrated in Figures 5.6 to 5.7 should also be presented. These will present an overall picture that is more consistent with how the water resource should be managed.

The specific questions relating to the designated categories will be the following:

- Water resource within designated management categories on all selected sampling dates?
- Water resource within designated ecological category on all selected sampling dates? Both toxicity and toxicant assessments can be displayed individually.
- Water resource within designated water use category on all selected sampling dates? Both toxicity and toxicant assessments can be displayed individually.

The data are obtained directly from the worksheet "One Point One Year Summary" for each monitoring point. The answer to the two questions "Ecological category 1 (Yes/No)?" and "Water Use Category 1? (Yes/No)" must be set to those associated with the designated management class.

Although not indicated in the following figures, value is added to the maps if the catchments represented by the monitoring points are also shown. This is consistent with the idea of presenting reasons for the choice of individual monitoring points. It provides the reader with a spatial indication of the extent of national coverage that is not possible to convey if only individual points are depicted. The relevant catchments can be indicated either by only depicting the outlines of those catchments or by highlighting (*e.g.* in colour) the relevant catchments on a map showing all catchments.

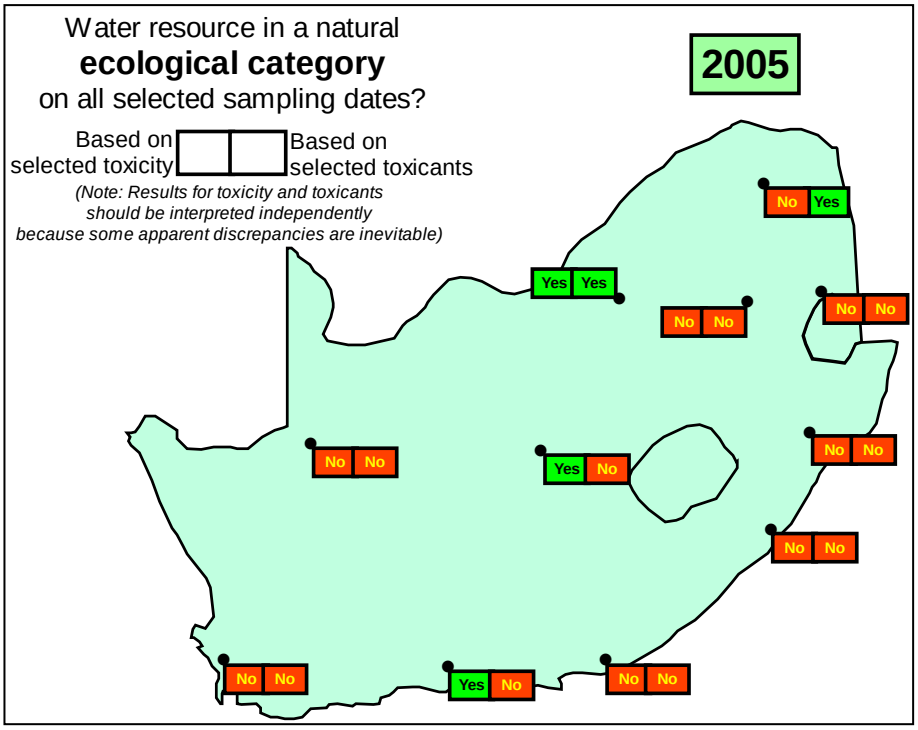


Figure 5.3. Illustration of map showing compliance with ecological category 1, based on toxicity and toxicants individually.

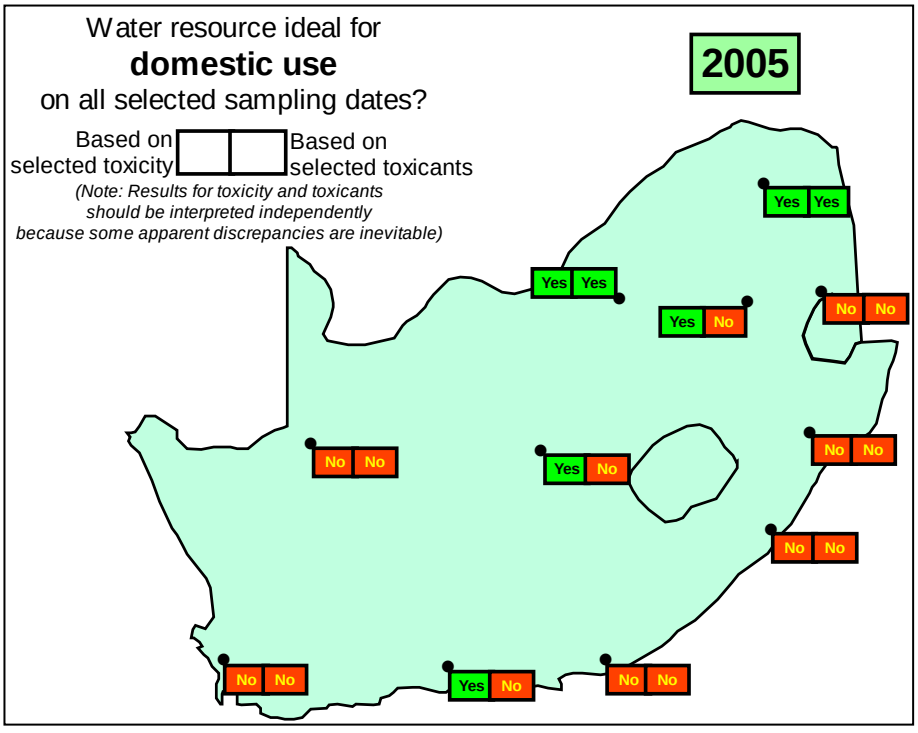


Figure 5.4. Illustration of map showing compliance with a domestic water use category, based on toxicity and toxicants individually.

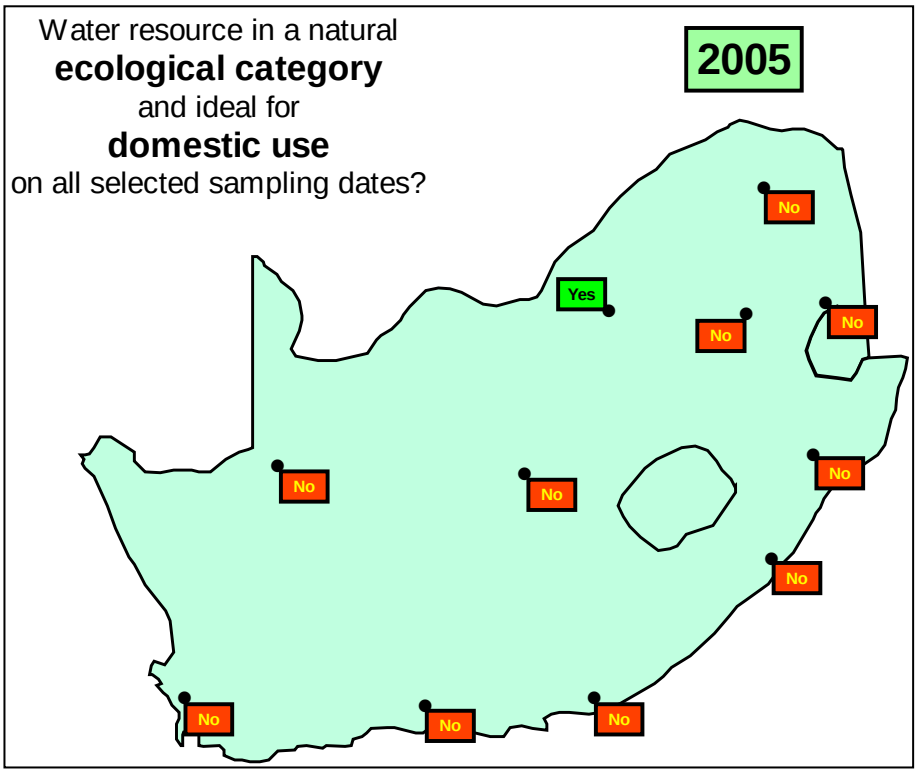


Figure 5.5. Illustration of map showing compliance with both an ecological category 1 and a domestic water use category 1.

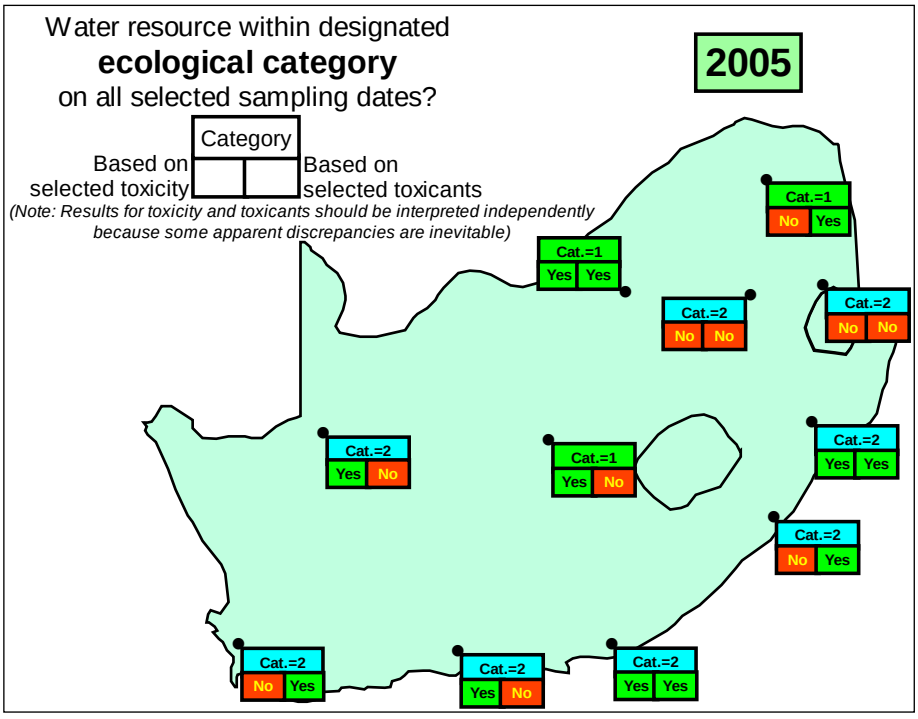


Figure 5.6. Illustration of map showing compliance with the designated ecological category, based on toxicity and toxicants individually.

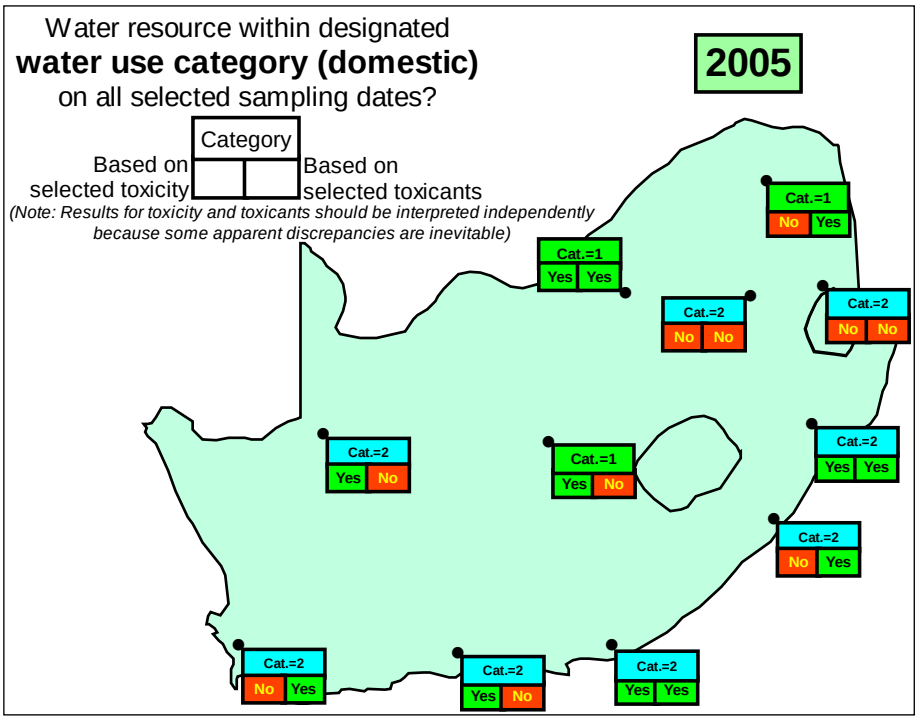


Figure 5.7. Illustration of map showing compliance with the designated water use category, based on toxicity and toxicants individually.

CHAPTER 6: QUALITY ASSURANCE AND QUALITY CONTROL ("HOW WELL")

This chapter describes how the overall quality of the NTMP can be assured.

6.1 INTRODUCTION

Quality assurance and quality control are two critically important processes that ultimately aim to ensure that the objectives of the NTMP are met. It is important to be clear on the meaning of the two terms "quality assurance" and "quality control". The following definitions are used for the NTMP:

Quality Assurance (QA). 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, and so on.

Quality Control (QC). 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.

Importantly, it should be noted that quality control is a quality assurance activity.

"Quality" simply means the degree of excellence that at least meets the needs of the target users. Although the highest quality is usually desirable, this is always associated with increased costs. The higher the quality achieved the higher the cost of achieving it. The challenge to QA and QC is to achieve the degree of excellence that is necessary to achieve the objectives of the NTMP at an affordable cost.

QA and QC are overarching activities that affect every aspect of the NTMP. The following sub-sections describe how QA and QC should be applied to the NTMP.

6.2 OVERALL FRAMEWORK

6.2.1 ISO 9001:2000

The ISO 9001:2000 [SANS, 2000] quality management system should be applied to the NTMP. The principles that underpin this system are as follows:

- *Customer focus*. The NTMP must all times understand the current and future needs of its target audience and strive at all times to meet these needs.

- *Leadership*. The complexity of the NTMP will require effective leadership to achieve its objectives.

- *Involvement of people*. The NTMP will involve the full cooperation of a very wide variety of role players. Lack of commitment from any of these could undermine the programme significantly.

- *Process approach*. The objectives of the NTMP can only be achieved efficiently when all activities are actively managed as an on-going process.

- *System approach to management*. The NTMP is not an isolated programme. Its success will depend on many related processes both inside and outside the Department. Successful management of the NTMP will require identifying these processes, understanding them and managing them appropriately.

- *Continual improvement*. A programme like the NTMP is never stagnant. It is dynamic and must adapt to its environment as that environment changes.

- *Factual approach to decision making*. The sensible analysis of data and information is critical to providing sound support for strategic decision-making.

- *Mutually beneficial supplier relationships*. Successful implementation of the NTMP will depend heavily on a wide variety of suppliers, ranging from those supplying equipment to those supplying data. "Win-win" relationships should preferably be set up with these role players.

The following sections outline a variety of approaches that apply the above principles.

6.2.2 Continual improvement

This is a critical principle of ISO 9001:2000. This should be achieved by broadly basing it on the cyclical "Plan, Implement, Check, Review" process associated with adaptive management. This cyclical approach aims at continual improvement over time.

- *Plan*: This refers to establishing and maintaining procedures that implement QA. It identifies objectives, allocates responsibilities and documents procedures.

- *Implement*: This refers to the communication of the plan, provision of resources, appointment of those accountable for the defined responsibilities, implementation of the procedures, and reporting on performance. Training, awareness creation and capacity creation in general is included here.

- *Check*: This involves monitoring the effectiveness of implementation of the QA plan through audits. Corrective actions (defined in the plan) must be applied when necessary.

- *Review*: At regular intervals the overall effectiveness of the NTMP and its QA procedures must be strategically reviewed. This usually occurs with the overall management review but can be more frequent.

In essence, QA must be planned carefully, implemented thoroughly, checked regularly for effectiveness and periodically reviewed.

6.2.3 Achieving NTMP objectives

At the highest level, the overall objective of QA and QC is to help ensure the objectives of the NTMP are achieved (see Chapter 1: Background). To be more specific on what this means, this section provides an interpretation of the individual phrases within the NTMP objectives from a QA perspective. A QA "review period" is also necessary. This refers to the interval between assessments of the degree to which each QA procedure or focus area has been successful in addressing the identified phrase in the objectives. At these times changes to individual procedures can be introduced if necessary.

"To measure, assess and report on a regular basis"

To address this, QA must ensure that the right data are measured. This includes the right variables at the designated number of times per year (sampling frequency) at the right places (monitoring point, depth of sampling, etc.). (See Chapter 3: Monitoring Framework.) The right analytical methods must be used (see Chapter 4: Sampling & Analysis) and the data must be managed correctly (see Chapter 5: Data Management and Reporting).

QA must also ensure that these data are correctly assessed. This includes ensuring the right procedures and appropriate guidelines are used (see Chapter 5: Data Management and Reporting).

QA must ensure that appropriate reports are written and distributed. This includes ensuring that the report format (written hardcopy, web site, etc.) is appropriate and that the report content is appropriate for the intended audience (see Chapter 5: Data Management and Reporting). The latter refers, for example, to the text (*e.g.* warnings or advice on interpretation of results, which is particularly important when reporting on toxicity) and the nature of the spatial presentation (use of maps, icons, annotations, etc.).

QA must ensure that reports are distributed on time to the target audience on an appropriately frequent basis (annual in this case).

QA Review period: Annual.

"on the status and trends of the nature and extent of,"

To address this, QA must ensure that the results present a suitable reflection of the status and trends of the toxicity and toxicants in the chosen inland surface water resources and the nature and extent of those factors.

QA Review period: 5 years (3 initially).

"first, the potential for toxic effects to selected organisms, and,"

To address this, QA must ensure that appropriate toxic effects are reported and the selected organisms are sensible.

QA Review period: 5 years (3 initially).

"secondly, selected potentially toxic substances"

To address this, QA must ensure that an appropriate set of toxicants is included in the NTMP.

QA Review period: 5 years (3 initially).

"in South African inland surface water resources"

To address this, QA must ensure gradual increasing coverage of appropriate water resources until the degree of national coverage is adequate within the financial constraints.

QA Review period: Annual.

"in a manner that will (A) support strategic management decisions"

To address this, QA must ensure, and carefully examine, the degree to which strategic (*i.e.* large in spatial and temporal scale) management decisions are truly being supported.

QA Review period: 5 years (3 initially).

"in the context of (1) fitness for use of those water resources"

To address this, QA must ensure that reports provide useful decision support in the context of domestic use of the resource.

QA Review period: 5 years (3 initially).

"and (2) aquatic ecosystem integrity, and"

To address this, QA must ensure that reports provide useful decision support in the context of the integrity (health) of the aquatic ecosystems in the resource.

QA Review period: 5 years (3 initially).

"(B) be mindful of financial and capacity constraints, yet,"

To address this, QA must continually focus on how execution of the NTMP can be made more cost-effective. A particular problem will be ensuring continuity of all those involved in the NTMP from samplers, through analysts to managers.

QA Review period: Annual.

"be soundly scientific."

To address this, QA must at all times ensure that good science is practised at all stages and levels of the NTMP. Design of the programme should be as objective as possible. Any review and possible re-design of the NTMP should also be objective. During implementation, samplers must take samples correctly and understand why particular procedures are necessary (like getting samples to laboratories within specified times). Assessment of results must be based on sound statistical techniques. Presentation of results must give a true and honest reflection of the results. They must also be understandable by the target audience. The possibility of misinterpretation must be minimised. These all contribute to overall good scientific practice.

QA Review period: Annual.

If attention is given to all the above QA issues at the prescribed intervals, the level of excellence associated with the implementation of the NTMP can be continually improved and maintained at a high standard.

6.2.4 Attitudes

Creating positive attitudes and pride in all role players can be a powerful quality assurance tool. And it can be relatively inexpensive to implement.

Perhaps the single issue that can most effectively contribute to an overall high level of excellence in NTMP implementation is the attitudes of those involved. Pride and ownership in all the role players (sampler, analysts, assessors, data managers, water resource managers, etc.) of their contribution to making the NTMP a high quality programme is also something that can potentially be achieved at relatively low cost.

Positive optimistic attitudes and team spirit in all concerned can potentially decrease the likelihood of quality problems arising from the lack of formal quality control (QC) procedures. The latter can easily become exorbitantly expensive. They can also be very demanding on practitioners who often feel the extra effort required by QC is not worth the apparent benefit.

It is not being suggested that creating positive attitudes should replace formal QC. However, when formal QC procedures are at risk of compromise (or even not even being implemented in the first place) because of lack of resources, positive attitudes may be all the NTMP has to fall back on.

A number of simple and very cheap mechanisms can be implemented to encourage such attitudes:

- Regularly communicate even small successes to those involved in data collection, assessment, data management, etc. This creates team spirit. The following are examples:
    - A particular dataset may have highlighted a previously unsuspected problem.
    - New monitoring points may have been added that further improve national coverage.
    - Positive comments by any water resource manager, or even the Minister, on the quality or usefulness of a report should be forwarded to all involved.
- Consider introducing simple reward mechanisms for work well done. This may involve a sampler who has not missed any scheduled sampling rounds over the past year. Rewards need not be monetary. They can be certificates or simply involve letting everyone involved know of the individual's achievements.

6.3 QA PROCEDURES

6.3.1 Sampling and sample transport

The importance of proper sampling procedures cannot be overemphasised. However, cost-effective quality control of sampling and sample transport activities is difficult. This is because these activities are often performed by a single person under circumstances where frequent supervision is not possible (or affordable). Nevertheless, the following single quality control activity should be implemented:

- A suitably qualified person can accompany the sampler/monitor on his/her rounds once a year. Sampling and sample transport procedures should be observed. If problems are evident they can be corrected immediately.

Because this measure cannot guarantee a sustained high level of quality, emphasis should be placed on other QA activities. These include effective training when the sampler/monitor is appointed. This should ideally be done in the field. Actual samples should be taken to ensure that each detail, and the reason for it, is clear to the sampler/monitor. Insulated containers in which the samples will be transported, including ice, should also be on hand and used.

6.3.2 Sample analysis

The analytical methods associated with the NTMP (*i.e.* the toxicity tests and the analysis of toxicants) are not trivial. (See Chapter 4: Sampling and Analysis for a description of the required tests and analyses.) They require particular expertise and experience to execute correctly. Developing effective quality assurance protocols is therefore particularly important.

6.3.2.1 Accreditation

The ultimate aim for the NTMP is that all laboratories performing these tests and analyses should be accredited for them. However, accreditation is time-consuming (to achieve and maintain) and expensive. Therefore, formal accreditation should not be given a high priority until the NTMP becomes established and a basic level of nationwide analytical capacity has been created.

6.3.2.2 QC for individual methods

Individual toxicity tests and analytical methods used for toxicants each have specific quality control measures documented with the method. These should be applied as prescribed.

6.3.2.3 QA for toxicity tests

Some QA and QC procedures for toxicity tests are described in Chapter 4: Sampling and Analysis. Furthermore, the DWAF D: RQS Analytical Laboratory also prescribes the following series of QA procedures specific to toxicity tests. These should be used as guidance in other participating laboratories.

- Sample holding times and temperatures must conform to the conditions described.
- Test organisms must be disease-free and positively identified to species.
- Fish must be handled carefully, using a small net to transfer them from one tank to another.
- Fishnets must be stored in a disinfectant. The nets must be rinsed thoroughly with dilution water before use.
- Laboratory temperature control equipment must be adequate to maintain recommended test water temperatures.

- Breeding and testing facilities must be separated to prevent contamination of fish cultures.
- Glassware, pipettes etc. must be calibrated.
- Glassware must be cleaned according to US EPA standards.
- Fish are susceptible to diseases and stress when subjected to sudden changes in temperature. Therefore, water temperatures should not differ by more than 3°C when fish are transferred from one tank to another.
- Instruments used for physical parameters must be calibrated according to the manufacturer's procedures.
- With toxicity samples, conductivity, pH, oxygen and free chlorine must be determined before a test start.
- All visible observations of the sample must be recorded such as colour, smell, algae present, etc.
- The quality of water used to prepare dilution water is extremely important and must be analysed for toxic metals and organic compounds when a problem is suspected.
- The nutritional quality of the food used in breeding must be adequate.
- Materials that come into contact with the sample or dilution water should be carefully chosen. Copper, galvanised material, rubber, brass and lead must not come into contact with holding or dilution water, or with the sample and test solutions.
- The water of the tanks must be kept clean, clear and practically odourless since water quality can affect the conditions and performance of fish, as well as test results. In a closed system as described, there is a build-up of materials that can be toxic to fish.
- The commonest cause of trouble in an aquarium is overfeeding. Uneaten food, which drops to the bottom of the tank, decomposes and sets up a chain of undesirable reactions.
- When purchasing fish it is essential to be quite certain that they come from a reliable source.
- Reference toxicant and temperature control charts should be used to document the health and sensitivity of the organisms used, data quality, and the overall laboratory performance.
- Methods must be well validated.
- A well-known statistical program such as Probit, Spearman-Kaber must be used to determine LC50 values or applicable / acceptable calculations / interpolation methods to determine LC50 values.
- Laboratory must participate in inter-laboratory exercises to enable comparison of results between laboratories.

6.3.2.4 QA for toxicant analyses

The DWAF D: RQS Analytical Laboratory prescribes the following series of QA procedures. These should be used as guidance in other participating laboratories.

- Sample management
    - Samples delivered for analysis should be inspected, labelled and registered.
    - Samples should be taken in glass containers, previously cleaned according to standard good laboratory practice.
    - Samples should be stored at approximately 4 °C until analysed.

- Sample preparation
    - Samples should be prepared for analysis according to the documented method appropriate for the required analysis.
    - Acceptable quality certified chemicals, solvents and reference compounds should be used in the preparation of the samples.

  - Blanks and fortified samples should be prepared in parallel with the samples in order to determine interferences and recovery efficiencies.
  - All containers, volumetric glassware, and equipment should be suitable and free from interfering contamination.

- Analysis
  - Gas chromatographs, mass spectrometers, liquid chromatographs, auto-samplers, etc. should be maintained in optimum operating condition.
  - Checks should be carried out prior to analyses to ensure that instrument conditions (temperatures, flow rates, etc.), columns, and all other equipment are suitable and optimised for the relevant analysis.
  - Checks should be done prior to analysis for repeatability (precision), accuracy, calibration validity and interferences.

- Reporting
  - Analytical reports should be audited.
  - Raw data should be checked for traceability (completeness of the paper trail).
  - Archives should be maintained for data retrieval / review.

- Competency
  - Laboratory analysts should undergo continued in-service training and evaluation to ensure competency.
  - Participation in accredited Inter-Laboratory Calibration Exercises should be done to evaluate and address credibility of results.

6.3.3 Data management

6.3.3.1 Introduction

Data management generally encompasses a wide range of activities:

- Registering the monitoring programme (monitoring points, sampling frequency, etc.),
- Receipt of analytical results (measured either in a laboratory or on-site),
- Capturing these results on WMS, and
- Making results available for subsequent processing (*e.g.* reporting).

The Water Management System (WMS) based at D:RQS (near Roodeplaat Dam north of Pretoria) will be the database used for all data associated with the NTMP.

6.3.3.2 Remote data capture

To a large extent, the current QA and QC procedures associated with WMS can be relied upon for the NTMP. One potential concern relates to the difficulties still being experienced in respect of remote data entry. Specifically, data capture directly onto WMS from a remote laboratory remains cumbersome and problematic. However, if a single central laboratory is created at D:RQS to address the specific analytical needs of the NTMP, then these problems are of no immediate concern. A laboratory at D:RQS should be able to interface directly with WMS, circumventing the remote entry problem. However, attention should still be given to the remote data capture problems to ensure that the problems have been solved when the number of monitoring points reaches the point where other laboratories are required (see Chapter 5: Data Management and Reporting, Section 5.2.4).

If other laboratories become involved in the NTMP before these problems are solved, then an interim solution should be used. Analytical data may be captured preferably electronically from a remote LIMS (or less preferably, manually) into a spreadsheet that is E-mailed to WMS data capturers who import it directly into WMS. The Record of Decision report that provides the background to this implementation manual should be consulted for recommended properties of such a spreadsheet [DWAF, 2005a].

6.3.3.3 QC in the NTMP assessment spreadsheet

Missing data (and data of dubious quality) can significantly affect the ability of the NTMP to achieve its objectives. More specifically, it can impact directly on the ability of water resource managers to make informed decisions.

The spreadsheet that was developed for annual reporting purposes contains a quality control worksheet providing the following:

- The percentage of sampling dates for which no data are available. It is proposed that the target (maximum) percentage be 10 %.
- The percentage of measurements relating to the ecological category not available. The potential total number of measurements (toxicity and toxicants) can be obtained from the actual number of dates on which samples were taken. It is proposed that the target (maximum) percentage be 5 %.
- The percentage of measurements relating to the water use category not available. This is calculated in the same way as the previous percentage. The target is also proposed to be 5 %.

Another important facility provided by the spreadsheet is the calculation of the confidence one can have in individual measurements. This in turn allows a calculation of confidence in the assessment of whether or not the resource is within the designated ecological category and water use category. Specifically what is calculated is the maximum probability (at 99 % confidence) that what is being reported (*i.e.* the assessment against the guideline) is incorrect. The calculation is based on estimates of coefficients of variation of the analytical methods being used. These coefficients of variation reflect a particular aspect of the quality of the measurement. The calculation of the maximum probabilities of reported values being incorrect is a powerful way of making use of this quality statistic (the coefficient of variation) to facilitate informed decision-making.

6.4 STAFF CONTINUITY

6.4.1 The problem

By its very nature, monitoring is repetitive and can continue for many years. It requires regular on-going focused effort to succeed. Role players like samplers and analysts establish a routine and consequently become increasingly efficient at what they do. However, when any such person is suddenly no longer available (through sickness, resignation, etc.) the continuity of the monitoring programme can be threatened. Unless there is someone who can take over from the primary person there is a significant risk of, at least, acquiring data of lesser quality or, at worst, missing data all together.

It is important to minimise the impact on monitoring of a person (responsible for a monitoring task) suddenly being unable to carry out that task.

6.4.2 Simple precautionary strategy

Each critical role (from sampler onwards) should be examined. To avoid the quality problems arising from a person (the "primary person") suddenly not being able to carry out their task, it must be ensured that there is someone (a "backup" person) who can take over from the primary person by the next time that particular task needs to be performed.

Two types of backup person could be identified:

- An existing member of staff can be used. That person must agree to take on the extra task and this should be made explicit in that person's formal job description.
- The task can be outsourced. However, training may still be required.

In either case, make it clear to the backup person that this responsibility will remain in effect:

- Permanently, or
- Until the original primary person resumes his / her post (in the case of, for example, temporary sickness), or
- Until a new person can be found and trained (in the case of, for example, resignation).

If mentoring by the primary person is necessary (*e.g.* for more complex tasks, like data assessment), then clearly there is no other option but to:

- Identify a backup person,
- Ensure appropriate training occurs (and re-training, if necessary), and
- Ensure that the necessary mentoring occurs on a sufficiently regular basis.

If formal mentoring is not necessary (*i.e.* a training course will suffice), then the issue becomes whether or not (a) one waits until a potential problem arises (*e.g.* the primary person resigns) or (b) one prepares for a problem in any case.

Therefore, as a precautionary approach, a backup person should be identified and trained as a matter of course, irrespective of the nature of the task. That is, do not wait until a potential problem arises.

6.4.3 Risk-based strategy

If the above precautionary approach is considered too onerous, then a simple risk-based calculation provides a way of quantifying the risks of quality problems arising from critical staff suddenly being unavailable. In this way, under some circumstances it may be acceptable to simply wait until a potential problem arises. The extra costs of training backup people can then be minimised by only focusing on those where the risk of quality problems are high.

Let $T_{interval}$ (weeks) = interval between tasks. For example, this may be based on the sampling frequency (say, 2 weeks) or even a reporting frequency (say, 52 weeks).

Let T_{train} (weeks) = time required to find and train a backup person for the task. Note that T_{train} refers to the total time from when the problem is identified to having the backup person sufficiently competent to perform the task in question. This may include time required to find and train the backup person. This requires synchronising the available time of trainers and trainee. Neither may be available at a moments notice to present or attend a training course. Complicated courses (*e.g.* involving some of the laboratory techniques) may require weeks to set up.

The following are two important scenarios:

- *Long training times*: If the time required to find and train the backup is equal to or longer that the interval between tasks (*i.e.* $T_{train} \geq T_{interval}$) then one cannot wait until a problem arises. A backup person must be identified immediately, trained and prepared to take over from the primary person at a moment's notice.
- *Short training times*: If T_{train} is very much less than $T_{interval}$ (*e.g.* only one week of training is necessary, or only a week is required to formalise outsourcing of the task, and the interval between tasks is, say, 8 weeks), then it may be cost-effective to wait until a problem occurs before appointing and training a backup person. However, it is important to realise that this approach is always associated with some risk of continuity (*i.e.* quality) problems: Risk of problems = $T_{train} / T_{interval}$. Note that the closer T_{train} gets to $T_{interval}$, the closer the risk of quality problems approaches one (*i.e.* absolute certainty of problems). It is proposed that a risk of 0.2 (or, equivalently, 20 %) may be acceptable. In effect, this means quality problems will occur once out of every five times the primary person is unable to perform his / her task.

In summary therefore, the risk-based approach involves ensuring a backup person is trained if the risk of problems = $T_{train} / T_{interval}$ is greater than 0.2 (although one in five times there will be a problem). As above, if mentoring by the primary person is required then training must occur anyway.

6.5 REVIEW

The overall cost-effectiveness of the chosen QA and QC procedures should be reviewed initially on an annual basis. It is important to remember that the ultimate aim is to ensure that the objectives of the NTMP are achieved. If changes are necessary to improve cost-effectiveness, these should be implemented in all participating organisations.

CHAPTER 7: ROLES & RESPONSIBILITIES ("WHO DOES WHAT")

This chapter highlights the responsibilities of each role player in the NTMP.

7.1 INTRODUCTION

Many individuals and organisations are involved in the implementation and operation of a national monitoring programme. Individual roles need to be properly executed for the overall programme to be successful. This chapter describes each individual role and associated responsibilities.

The roles cover the whole range from sampler/monitor to national policy maker. This has been done to ensure that all role players understand where they fit into the overall picture. This should facilitate buy-in to the process by all involved. This can be regarded as contributing to quality assurance and hence, ultimately, sustainability of the programme.

7.2 NATIONAL POLICY MAKER

7.2.1 Responsibilities

The national policy maker receives annual reports from the programme manager. These reports are the "information products" that address the specific objectives of this monitoring programme. It is the responsibility of the national policy maker to use this information for the intended objectives.

A Minister is generally responsible for the powers and functions assigned to him/her by the President. As a Member of Cabinet, he or she is accountable to Parliament for the exercise of these powers and the performance of their functions. A Member of Cabinet must (a) act in accordance with the constitution and (b) provide Parliament with full and regular reports concerning matters under their control.

The following extract from the National Water Act summarises in general terms the ultimate responsibility of the Minister of Water Affairs and Forestry. The Director General, the Departmental Management Committee (MANCO) and the Water Resources Functional Management Committee (WRFMC) are expected to act accordingly.

"Sustainability and equity are identified as central guiding principles in the protection, use, development, conservation, management and control of water resources. These guiding principles recognise the basic human needs of present and future generations, the need to protect water resources, the need to share some water resources with other countries, the need to promote social and economic development through the use of water and the need to establish suitable institutions in order to achieve the purpose of the Act. National Government, acting through the Minister, is responsible for the achievement of these fundamental principles in accordance with the Constitutional mandate for water reform. Being empowered to act on behalf of the nation, the Minister has the ultimate responsibility to fulfil certain obligations relating to the use, allocation and protection of and access to water resources."

7.2.2 Typical role player

Minister of Water Affairs and Forestry, Director General, Departmental Management Committee (MANCO), Water Resources Functional Management Committee (WRFMC).

7.3 PROGRAMME MANAGER

Prescribed procedures: All chapters in this manual

7.3.1 Responsibilities

The function is to facilitate, coordinate and manage the nationwide implementation of the monitoring programme so that the objectives are achieved (see Chapter 2: National Implementation). The programme manager will typically represent the Department in negotiations with regional and local parties.

The programme manager will need to be familiar with all aspects of toxicity monitoring and should be able to provide technical and managerial advice to role players at all levels. The programme manager must ensure effective and efficient transfer of knowledge and experience gained by those involved in the programme.

To the extent considered necessary to keep national target users adequately informed, the programme manager must provide reports and feedback at an appropriate frequency.

7.3.2 Typical role player

A person from the Department of Water Affairs and Forestry (DWAF) with good managerial capabilities and a sound technical knowledge of biotoxicology.

7.4 REGIONAL MANAGER

Prescribed procedures: All chapters in this manual

7.4.1 Responsibilities

The function is to facilitate, coordinate and manage the regional implementation of the monitoring programme to the degree prescribed by the programme manager. Depending on local circumstances, the regional manager's tasks may simply involve managing samplers/monitors and ensuring samples reach designated laboratories. If such laboratories are local laboratories, then regional management may also include interfacing with these to ensure analyses are performed on time and that results are transmitted to the central database at regular intervals.

The regional manager, because of his or her specialised local knowledge, will usually also be expected to bring new potential NTMP priority areas in their region to the attention of the programme manager.

7.4.2 Typical role player

A person from a regional office of the Department of Water Affairs and Forestry (DWAF) or a catchment management agency with good managerial capabilities.

7.5 TARGET USERS

7.5.1 Responsibilities

The target users receive the same annual reports from the programme manager that are sent to the national policy maker. Government departments should use the products of the monitoring programme to contribute constructively to strategic national decisions in the context of the fitness for use of water resources and protection of aquatic ecosystems.

All target users must communicate their concerns, comments and suggestions to the programme manager. It is the responsibility of the target users to become involved in the monitoring programme to the extent necessary to ensure that the programme produces information products that adequately address the objectives of the programme.

7.5.2 Typical role player

Any organisation identified as a primary or secondary target user (see Section 5.3.2).

7.6 QUALITY MANAGER

Prescribed procedures: Chapter 6: Quality Assurance & Quality Control

7.6.1 Responsibilities

The quality manager is responsible for ensuring that the objectives of the NTMP are achieved by:

- Ensuring QA & QC procedures are applied consistently nationwide,
- Ensuring that the principles of ISO 9001:2000 [SABS, 2000] are applied,
- Applying a continual improvement (adaptive management) approach,
- Encouraging positive attitudes of all involved,
- Ensuring staff continuity is maintained, and
- Periodically reviewing quality assurance procedures.

7.6.2 Typical role player

Staff member of Directorate: Resource Quality Services

7.7 DATA MANAGER

Prescribed procedures: Chapter 5: Data Management & Reporting

7.7.1 Responsibilities

The data manager is responsible for:

- Registering the monitoring programme (monitoring points, monitoring frequency, etc.),
- Managing sampling (printing schedules for monitors and laboratories, printing sample tags, etc.)
- Receipt of analytical results (measured either in a laboratory or on-site),
- Capturing these results on the Water Management System, and
- Making the data available for subsequent processing.

7.7.2 Typical role player

Directorate: Resource Quality Services.

7.8 ANALYST

Prescribed procedures: Chapter 4: Sampling & Analysis

7.8.1 Responsibilities

The analyst is responsible for:

- Receiving samples from the sampler/monitor,
- Storing samples appropriately,
- Analysing for the defined monitoring variables within prescribed times, and
- Transmitting results to the Water Management System (WMS) as soon as possible.

7.8.2 Typical role player

Laboratory at Directorate: Resource Quality Services or other designated laboratory.

7.9 SAMPLER/MONITOR

Prescribed procedures: Chapter 4: Sampling & Analysis

7.9.1 Responsibilities

The sampler/monitor is responsible for:

- Travelling to the designated monitoring points at the agreed times,
- Taking the samples at the designated places,
- Preserving the samples (only if necessary),
- Marking the sample containers with the time, date and other monitoring point identification, and
- Ensuring the samples are delivered in the appropriate insulating containers either (a) directly to the nearest participating laboratory or (b) to an appointed courier company for transport to the laboratory.

7.9.2 Typical role player

Participating laboratory, Catchment Management Agency or a regional Department of Water Affairs and Forestry officer, water board, local authority or local officials of the Department of Environmental Affairs and Tourism or Department of Health who have undergone adequate training in the sampling methods necessary for this monitoring programme.

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APPENDIX: BIOTOXICOLOGY OVERVIEW

This chapter provides a general overview of the field of biotoxicology (*i.e.* toxicants and their effects on organisms).

A.1 INTRODUCTION

Biotoxicology is the qualitative and quantitative study of the adverse effects of chemical pollutants and other anthropogenic materials on living organisms. The current national monitoring context only concerns itself with a subset of this extremely broad field.

Aquatic toxicology, in particular, is a multidisciplinary science that integrates expertise from biology (biological structure, composition and function of aquatic ecosystems) and relates to establishing the concentration of toxicants in the aquatic environment. Establishing the concentration requires expertise in the distribution, transformation and fate of toxicants. This is a function of physical factors (*e.g.* solubility, volatility, etc.), chemical factors (hydrolysis, photolysis, oxidation and reduction, etc.) and biological factors (*e.g.* bioaccumulation, biotransformation, biodegradation) [Rand and Petrocelli, 1995].

An important basic principle of toxicology is that no chemical is completely safe and no chemical is completely toxic [Rand and Petrocelli, 1995]. Even apparently harmless chemical substances can have toxic effects when taken up by an organism in sufficient amounts. Conversely, uptake of small amounts of some toxic chemicals may result in no apparent toxic effect. Typically the effect on an organism depends on (a) the quantity (or concentration) of the chemical to which the organism is exposed, (b) the nature of the exposure and (c) the duration of that exposure.

A.2 TOXICITY

A.2.1 The nature of toxicity

For a substance to produce a toxic effect on aquatic or other organisms, the following must occur.

1) The substance must come into contact with the organism.
2) It must react with an appropriate receptor site on the organism (a) at a high enough *concentration*, and (b) for a *sufficient length of time*.

Toxicants can affect organisms on land, in the air and in natural waters. However, the current context is restricted to those organisms, including humans, which use or rely on South African fresh and estuarine water resources.

This section addresses toxic effects only. It does not necessarily try to link specific toxicants with these effects. However, it is sometimes possible to use specific toxicity tests to detect specified toxicants, such as estrogen mimics and some heavy metals.

A.2.1.1 Organisms

The types of organisms of interest would typically include at least those reasonably associated with the various water uses for which water quality guidelines have been developed (even if not for toxicants *per se*). The following table suggests such an association.

Table A.1. Target organisms associated with the standard water uses.

Water use	Target organisms
Domestic	Humans
Recreational	Humans
Industrial*	Humans
Aquatic ecosystem organisms	Fish, invertebrates, birds, mammals, plants
Agriculture - irrigation	Humans, plants
Agriculture – livestock watering	Mammals
Agriculture – aquaculture	Fish, reptiles, plants

* Equivalent to domestic use in the current context.

Human health is obviously a primary concern in the context of national toxicity monitoring. However, impacts on certain animals in agriculture (livestock, fish, etc.) are also important considerations as are potential impacts on general ecosystem health. Therefore, the range of organisms that can potentially exhibit toxic effects is very large.

A.2.1.2 Toxic effects

The reader is referred to the glossary for definitions of many of the terms used here. To many of the above organisms, the nature of the toxicity can be reported in the following three contexts.

Short-term versus long-term effect: One broad classification of toxic effect relates to the time required for the effect to manifest itself. "Short-term effect" can refer to those toxic effects that manifest relatively quickly (within hours or days). On the other hand "long-term effects" can refer to those that take longer to manifest. These terms are also commonly referred to as acute and chronic effects.

Reporting toxic effects as short-term or long-term is useful to a water quality manager. If water from a particular river reach exhibits acute toxicity to fish, this serves as an immediate red flag in respect of the general health of fish populations and diversity in that area. If that river reach is being monitored by the national biomonitoring programme, this toxicity information would be useful to compare with their observations. In this way the NTMP would complement other monitoring programmes.

Lethal or sub-lethal: The next most obvious distinction that one can make in respect of the nature of toxic effects is whether the effect is lethal or not (*i.e.* "sub-lethal"). Lethality is usually a short-term effect.

Type of sub-lethal effect: If the effect is sub-lethal, then the type of effect can be reported. Sub-lethal effects include a very wide variety of adverse responses to exposure to toxicants. Typical sub-lethal effects in aquatic organisms include the following [Rand and Petrocelli, 1995]:

- *Biochemical and physiological effects.* These include effects related to enzyme inhibition, clinical chemistry, haematology and respiration.
- *Behavioural effects.* Typical behavioural effects include locomotion and swimming, attraction-avoidance, predator-prey relationships, aggression and territoriality and learning.
- *Histological effects*. These relate to structure and chemical composition of the animal or plant tissues as related to their function.

Some biochemical and physiological effects can also apply to humans and other mammals. These include mutagenicity, carcinogenicity, tumour promotion, teratogenicity, oestrogenicity and endocrine disruption.

A.2.2 Factors affecting the extent

The degree of toxicity (*i.e.* the extent to which the toxic effect manifests itself) is determined by various properties of the toxicant and complex interactions between exposure and organism-specific factors. Some are illustrated in Figure A.1. Note that the degree of toxicity depends directly on the concentration of the toxicant. Factors determining this concentration and speciation are discussed above.

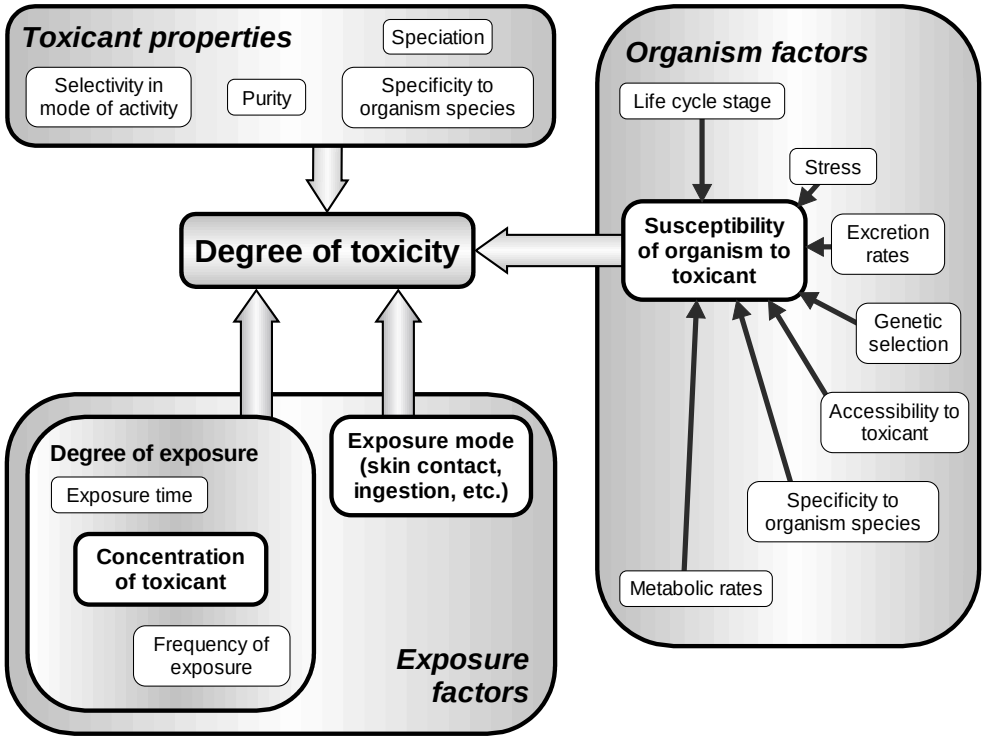


Figure A.1. Some toxicant properties, exposure and some organism factors determining the degree of toxicity.

A.2.3 Measurement of the extent

"Toxicity tests" (see glossary) measure directly the degree (*i.e.* extent) of toxicity on specific target organisms. Slabbert *et al.* (1998a) should be consulted for more detailed information on various toxicity test methodologies. A wide variety of scenarios and issues are relevant when choosing an appropriate toxicity test.

In situ versus in laboratory: Some tests are designed to take place in the natural water itself while others require water samples to be transported to a laboratory where the test takes place. Most single-species tests are conducted in the laboratory. Such tests are convenient because they allow a much greater degree of control than those performed in the field. However, the usefulness of tests done in the laboratory will depend on the criteria used to choose the organism. One limitation of such tests is that the effects observed in the laboratory may not occur in exactly the same way in the natural environment.

Toxicity tests performed in the natural environment are more likely to determine effects that are more representative. However, difficulties are created by natural variability in the environment. This can make it difficult to establish that an observed effect is really due to a chemical toxicant.

In the current context of long term monitoring of water resources, the most applicable tests are those in which the test organism is directly exposed to the water with no dilution. However, it is also possible to concentrate water samples, thus increasing the concentration of the toxicants to which test organisms are exposed. This can increase the detection potential of a test.

Variability is a significant issue even in laboratory toxicity testing. This applies to both control samples and test samples and therefore creates some degree of uncertainty in reporting. Generally, a lethal effect requires at least a 10% effect for the effect to be regarded as lethal. Sub-lethal effects typically require a 20% effect.

Short-term versus long-term effect: Some tests are specifically designed to measure effects over the short term and others over the long term. Common short-term tests include measuring fish and invertebrate lethality or algal growth over a fixed period of time [Rand and Petrocelli, 1995]. Long-term tests typically can involve exposing organisms to the toxicant over an entire reproductive cycle or part of it and measuring growth and reproduction [Rand and Petrocelli, 1995].

Lethal versus sub-lethal: Lethality can be measured in terms of the percentage of a selection of test organisms that die within the test period. The degree to which sub-lethal effects manifest themselves is also usually reported quantitatively, most commonly as a percentage effect. In particular, this usually refers to the percentage of organisms (or activity) affected.

A.3 TOXICANTS

A.3.1 The nature of toxicants

Toxicants in general can occur in a very wide variety of physical forms, including dusts, fumes, mists, vapours and gases, liquids and solids [Sax, 1974]. In the current context, toxicants are confined to chemical pollutants capable of exhibiting a toxic effect.

Monitoring to establish the specific source of the toxicants is explicitly not included in the monitoring objectives of the NTMP. However, for the sake of completeness, Table A.2 summarises a few typical sources of some toxicants. Organic compounds comprise the widest variety of toxicants, some classes of which also appear in the table.

Table A.2. Examples of potential sources of various toxicants in natural waters.

Toxicant	Typical sources
Heavy metals	Mining industry, chemical industry, tanning
Inorganics	Mining industry
Pesticides	Pesticide manufacture and formulation; Agriculture
Petroleum products	Petroleum industry
Petrochemicals	Petrochemical industry
Surfactants	Household aqueous waste, industrial laundering and other cleansing operations
Pharmaceuticals	Pharmaceutical industry, agriculture, hospitals

The following subsections describe briefly some of the classes of chemical pollutants of potential concern. It might be noted that these classes are not mutually exclusive (as very roughly illustrated in Figure A.2). That is, some specific toxicants may appear in more than one class. Nevertheless, these classes are defined and used here because they are classifications that are in common use. Examples of toxicants are given for each class.

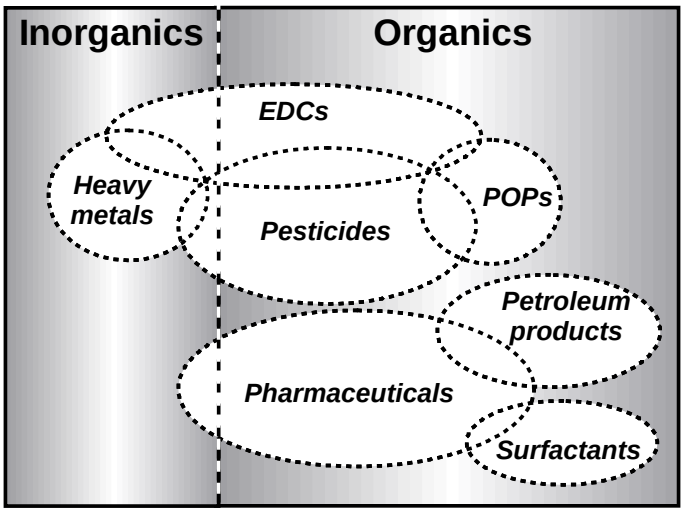


Figure A.2. Illustration of some of the overlaps between some classes of toxicants (EDCs = Endocrine Disrupting Compounds, POPs = Persistent Organic Pollutants).

A.3.1.1 Inorganic substances

Heavy metals are defined as metallic elements with atomic number greater than 20 (*i.e.* that of calcium) [World Bank, 1999]. These heavy metals are highlighted in the periodic table (Figure A.3).

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	**Sc**	**Ti**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Ga**	**Ge**	As	Se	Br	Kr
Rb	**Sr**	**Y**	**Zr**	**Nb**	**Mo**	**Tc**	**Ru**	**Rh**	**Pd**	**Ag**	**Cd**	**In**	**Sn**	**Sb**	Te	I	Xe
Cs	**Ba**	*L*	**Hf**	**Ta**	**W**	**Re**	**Os**	**Ir**	**Pt**	**Au**	**Hg**	**Tl**	**Pb**	**Bi**	**Po**	At	Rn
Fr	**Ra**	*A*															
		L	**La**	**Ce**	**Pr**	**Nd**	**Pm**	**Sm**	**Eu**	**Gd**	**Tb**	**Dy**	**Ho**	**Er**	**Tm**	**Yb**	**Lu**
		A	**Ac**	**Th**	**Pa**	**U**	**Np**	**Pu**	**Am**	**Cm**	**Bk**	**Cf**	**Es**	**Fm**	**Md**	**No**	**Lr**

Figure A.3. The elements typically regarded as "heavy metals" (highlighted).

Most of the lanthanides (the so-called "rare earths", from La to Lu) and the actinides (from Ac to Lr) are particularly uncommon and not likely to be relevant in a national monitoring programme. However, some, like uranium (U), are potential toxicants that could feasibly occur in South African aquatic environments. However, it might be reasonably suggested that uranium, and other radioactive elements, are better dealt with in the national radioactivity monitoring programme. Accordingly, should "heavy metals" ever be considered to be within the scope of the NTMP, a more operational definition would then include the metals indicated in Figure A.4.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	**Sc**	**Ti**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Ga**	**Ge**	As	Se	Br	Kr
Rb	**Sr**	**Y**	**Zr**	**Nb**	**Mo**	**Tc**	**Ru**	**Rh**	**Pd**	**Ag**	**Cd**	**In**	**Sn**	Sb	Te	I	Xe
Cs	**Ba**	*L*	**Hf**	**Ta**	**W**	**Re**	**Os**	**Ir**	**Pt**	**Au**	**Hg**	**Tl**	**Pb**	**Bi**	**Po**	At	Rn
Fr	**Ra**	*A*															

L	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
A	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Figure A.4. Operational definition of the 44 "heavy metals" for the NTMP (highlighted).

In the current context, elemental forms of the metals (*i.e.* the pure uncharged form not chemically bound to any other element) are not usually an issue. One exception is mercury, Hg, which is significantly soluble in water in its elemental form, Hg^0, as well as occurring in other charged and neutral forms. Most metals tend to exhibit their toxicity when they occur as ions. Metals can occur in aquatic environments in various forms.

- They can be dissolved hydrated cations (*i.e.* the positively charged metal ion surrounded by water only, like Fe^{2+} or Cd^{2+}).

- The dissolved cation may bind to other dissolved inorganic or organic compounds forming "complexes" or adsorb onto the charged surfaces of solids. These dissolved complexes may be negatively charged (like $Fe^{II}PO_4^-$), positively charged (like $HgCl^+$) or neutral (like $PbCO_3$). The degree of binding depends on the relative concentrations, the inherent strength of the bonds formed and various properties of the water such as pH, oxidation potential and salinity.

- Some heavy metals commonly occur as negatively charged ions, like molybdate ($Mo^{III}O_4^{2-}$) or chromate ($Cr^{III}O_4^{2-}$), which can bind to other positively charged metal ions or surfaces.

- Some heavy metals, like mercury (Hg), can also occur as organometallic compounds, like methylmercury (CH_3Hg).

- Many metals are very stable in precipitated (solid) forms, particularly in suspended solids and sediments. For example, iron and manganese form various oxides and oxyhydroxides. Unless the conditions (*e.g.* of pH and reduction potential) of the aquatic environment change, iron and manganese can remain in some solid forms indefinitely.

It might finally be noted that the metal aluminium (Al), although strictly not a heavy metal, can also be toxic.

Other inorganic compounds include many that are anions (*i.e.* have a negative charge). These are typically comprised of non-metallic elements, as indicated in the following figure.

H																	He
Li	Be											**B**	**C**	**N**	**O**	**F**	Ne
Na	Mg											Al	**Si**	**P**	**S**	**Cl**	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	**As**	**Se**	**Br**	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	**Te**	I	Xe
Cs	Ba	*L*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	**At**	Rn
Fr	Ra	*A*															
		L	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
		A	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Figure A.5. Non-metallic elements present in many inorganic compounds.

It should be noted that although carbon (C) is the defining element for something being "organic", when in its higher oxidation states it is typically regarded as an inorganic substance, *e.g.* as in carbonate (CO_3^{2-}), cyanide (CN^-) or cyanate (CNO^-).

Typical examples of inorganic substances that are known to exhibit significant toxicity to certain organisms are the following:

Cyanide (CN^-), ammonia (NH_3), chloride (Cl^-), arsenic (usually as arsenate, $As^VO_4^{3-}$), borate ($B(OH)_4^-$), fluoride (F^-), nitrate (NO_3^-), nitrite (NO_2^-), selenate (SeO_4^{2-}), sulfide (S^{2-}).

Like metals, the inorganic ions exist in the aquatic environment in various forms. These include dissolved forms that are unbound to anything (except the surrounding water) or complexed to metal cations, adsorbed onto solid surfaces or precipitated.

A.3.1.2 Organic compounds

Organic compounds all contain carbon (C) in one or more of its more reduced oxidation states and usually hydrogen (H) and sometimes other elements like oxygen (O), nitrogen (N) and sulfur (S). The number of organic compounds is enormous. They are used in a very wide variety of applications, many of which result in their entering the natural environment. Many degrade naturally, albeit at vastly different rates. Many can produce breakdown products that are themselves toxic.

"Organic compounds" is such a broad classification that it is more appropriate to deal with the various classes separately. This is done in the following subsections.

A.3.1.3 Endocrine disrupting compounds

Endocrine disrupting compounds (EDCs) are chemicals that mimic natural hormones, inhibit the action of natural hormones or alter the normal regulatory function of the immune, nervous or endocrine systems. These effects can manifest at extremely low concentrations creating particular challenges for their analysis. Known EDCs are mainly of organic compounds but some heavy metals also have endocrine disrupting properties. The study of EDCs is a relatively new field worldwide.

A.3.1.4 Pesticides

Pesticides are 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 [World Bank, 1999]. They are usually organic compounds, but may also contain inorganic substances.

The major chemical groups that are formulated (by combining active ingredients) include [World Bank, 1999]:

- Insecticides (organophosphates, carbamates, organochlorines, pyrethroids, biorationals and botanicals).
- Fungicides (dithiocarbamates, triazoles, morpholines, pyrimidines, phthalamides and inorganics).
- Herbicides (triazines, carbamates, phenyl ureas, phenoxy acids, bipyridyls, glyphosates, sulfonyl ureas, amide xylenols and imidazole inones).
- Rodenticides (coumarins).

The synthesis of the active ingredients used in pesticides involves chemical manufacturing processes. Internationally, the major chemical groups manufactured include [World Bank, 1999]:

- Carbamates and dithiocarbamates (carbofuran, carbaryl, ziram and benthiocarb).
- Chlorophenoxy compounds (2,4-D, 2,4,5-T and silvex).
- Organochlorines (dicofol and endosulfan).
- Organophosphorous compounds (malathion, dimethoate, phorate and parathion methyl).
- Nitro compounds (trifluralin).
- Miscellaneous compounds such as biopesticides (like *Bacillus thuringiensis* and pherhormones), heterocycles (like atrazine), pyrethroids (like cypermethrin) and urea derivatives (like diuron).

The World Bank (1999) urges that special attention be given to certain restricted substances. These include hexachlorobenzene, toxaphene, chlordane, aldrin, DDT, mirex, dieldrin, endrin and heptachlor.

A.3.1.5 Persistent Organic Pollutants (POPs)

The Stockholm Convention

In 2001 a convention was signed in Stockholm, Sweden, with the objective "to protect human health and the environment from persistent organic pollutants". This convention proposed measures to reduce or eliminate releases from intentional and unintentional production and use, and from stockpiles and wastes. The signatories were encouraged, among other things, to monitor POPs in humans and the environment, as well as their effects. To be classified as a POP, a substance must be highly persistent, mobile and toxic [Ritter *et al.*, 1995].

The individual substances are listed in the following table.

Table A.3. Persistent organic pollutants addressed by the Stockholm Convention.

Chemical	Pesticide	Industrial chemical	Byproduct
Aldrin	Yes		
Chlordane	Yes		
Dieldrin	Yes		
Endrin	Yes		
Heptachlor	Yes		
Mirex	Yes		
Toxaphene	Yes		
DDT	Yes		
Hexachlorobenzene	Yes	Yes	Yes
PCBs (Polychlorinated Biphenyls)		Yes	Yes
Dioxins (Polychlorinated dibenzo-p-dioxins, PCDD)			Yes
Furans (Polychlorinated dibenzo-p-furans, PCDF)			Yes

Pesticides

The following information on the individual substances is taken from Ritter *et al.*, 1995.

- *Aldrin* is toxic to humans, the lethal dose estimated as 83 mg/kg body weight. It is highly toxic to laboratory mammals. It has low toxicity to plants and its toxicity to aquatic organisms is variable with aquatic insects the most sensitive group of invertebrates. Aldrin is readily metabolised to dieldrin in plants and animals though binds strongly to soil particles.

- *Chlordane* is semi-volatile and binds readily to aquatic sediments and bioconcentrates in the fat of organisms. Humans exhibit a wide variety of symptoms to chlordane exposure. Selected studies on aquatic organisms reported large differences within and between species. This was attributed to differences in water temperature and sediment loadings.

- *Dieldrin* is toxic to humans, the lethal dose estimated as 10 mg/kg body weight. It is also highly toxic to laboratory mammals. It has low toxicity to plants and variable acute toxicity to aquatic invertebrates and birds. Its persistence and lipid solubility creates the conditions for dieldrin to bioconcentrate and biomagnify in organisms.

- *Endrin* is chemically closely related to dieldrin. Endrin is toxic to humans (estimated lethal dose 100 mg/kg body weight) though its carcinogenicity to humans cannot yet be classified. It is metabolised rapidly in animals with very little accumulating in fat compared with compounds of similar structure. It is highly toxic to fish.

- *Heptachlor* is classified as a possible human carcinogen and has been implicated in the decline of several wild bird populations. It bioconcentrates in organisms.

- *Mirex* is very resistant to breakdown and has been shown to bioaccumulate and biomagnify. There is little information on its effects on humans. However, based on evidence involving laboratory mammals, it is classified as a possible human carcinogen. Crustaceans are the most sensitive aquatic organisms.

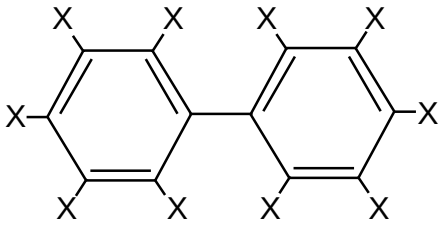
- *Toxaphene* is classified as a possible human carcinogen. It is essentially non-toxic to plants and is likely to bioconcentrate in animals.

- *DDT* is classified as a possible carcinogen. It is highly toxic to fish. It is not highly toxic to birds but affects reproduction through egg shell thinning. DDT and related compounds are very persistent in the environment.

- *Hexachlorobenzene* is a fairly volatile and very persistent fungicide which readily bioconcentrates in the fat of organisms. It has low acute toxicity, though high chronic toxicity, to mammals and is unlikely to cause direct toxicological effects in aquatic animals at or below the saturation levels in water (about 5 µg/ℓ).

Polychlorinated Biphenyls (PCBs)

The PCBs comprise many related compounds ("congeners") with the following basic structure:



$C_{12}H_{(10-n)}Cl_n$ n = 1,..,10 X = H or Cl

Figure A.6. Molecular structure of Polychlorinated Biphenyls (PCBs).

As indicated in the figure, the number of chlorine atoms can vary from 1 to 10. When the number of chlorine atoms is less than 10, they can be distributed over the biphenyl structure in a number of ways. Theoretically, this gives rise to 209 congeners, however only about 130 of these are likely to occur in commercial products [UNEP, 1999]. Commercial PCBs are a mixture of 50 or more PCB congeners [UNEP, 1999]. The following extract is taken from UNEP (1999):

"*PCBs can be transported long distances, and have been detected in the furthest corners of the globe, including places far from where they where manufactured or used. While manufacture of PCBs has reportedly ceased, the potential or actual release of PCBs into the environment has not, since significant quantities of PCBs continue to be in use or in storage.*

The likely extended period of these continuing uses, and the persistence of PCBs once released into the environment together mean that PCBs could pose a threat for decades to come."

Dioxins and furans

Dioxins and furans have very similar structures and properties [Ritter *et al.*, 1995]. They are unwanted by-products of various technological processes, but were never produced commercially and have no intended use [Goldman and Tran, 2002]. Of the 210 dioxins and furans, 17 contribute most significantly to the toxicity of complex mixtures [Ritter *et al.*, 1995]. They are very stable and persistent and their properties favour long-range transport. One particular dioxin has been extensively studied, referred to as TCDD. It is carcinogenic to humans.

Properties of POPs

Most of the POPs are characterised by properties that favour long-range transport in the environment. This is commonly via atmospheric routes. This means that they can occur in the environment far from their original sources. The following table summarises some relevant properties. The Chemical Abstracts Substance number is a unique identifying number that overcomes the problems caused by many substances having multiple names.

- **Log K_{OC}.** This is the logarithm of the soil/sediment partition or sorption coefficient. It provides an indication of the tendency of a chemical to partition between particles containing organic carbon and water [Ritter *et al.*, 1995].
- **Log K_{OW}.** This is the logarithm of the octanol-water partition coefficient. It has been shown to be linearly correlated with the log bioconcentration factors in aquatic organisms [Ritter *et al.*, 1995].
- **Water solubility.** This is the maximum concentration of the substance in water at the given temperature.
- **Vapour pressure.** This is a measure of the volatility of the substance, or driving force for the substance to become gaseous.

Table A.4. Properties of persistent organic pollutants [mainly from Ritter *et al.*, 1995].

Chemical	Chemical Abstracts Substance No.	Log K_{OC}	Log K_{OW}	Water solubility (µg/ℓ)	Vapour pressure (mm Hg at 20ºC)
Aldrin	309-00-2	2.6, 4.7	5.2-7.4	17-180 (25 ºC)	$2.13x10^{-5}$
Chlordane	57-74-9	4.6-5.6	6.0	56 (25 ºC)	10^{-6}
Dieldrin	60-57-1	4.1-4.6	3.7-6.2	140 (20 ºC)	$1.78x10^{-7}$
Endrin	72-20-8		3.2-5.3	220-260 (25 ºC)	$7x10^{-7}$
Heptachlor	76-44-8	4.4	4.4-5.5	180 (25 ºC)	$3x10^{-4}$
Mirex	2385-85-5		7.2[1]	85 (? ºC)[1]	$7x10^{-7}$ (25 ºC)
Toxaphene	8001-35-2	3.2	3.2-5.5	550 (20 ºC)	$6.7x10^{-6}$ (? ºC)[1]
DDT	50-29-3	5.1-6.3	4.9-6.9	1.2-5 (25 ºC)	
Hexachlorobenzene	118-74-1	2.6-4.5	3.0-6.4	40 (20 ºC)	$1.09x10^{-5}$
PCBs			4.3-8.3	0.001-5500 (? ºC)	$2.3x10^{-7}$ – 0.019
Dioxins			4.8-8.2	0.00074 – 417 (? ºC)	
Furans			5.4-8.0	0.0012 - 14.5 (? ºC)	

[1] Jia *et al.*, 2004.

A.3.1.6 Petroleum products

Petroleum products comprise another extremely wide range of chemicals that occur in, or are derived from, petroleum. Petroleum itself consists of a range of hydrocarbons usually classified in terms of the number of carbon atoms in the molecules, as indicated in Table A.5.

Hydrocarbons are typically hydrophobic. This means they do not dissolve in water like heavy metals and charged organic compounds. Their concentrations in the aquatic environment are therefore usually low. However, such compounds usually have an affinity for fatty tissues and therefore bioaccumulate in animal tissues.

Table A.5. Petroleum constituents [Morrison and Boyd, 1987].

Fraction	Distillation temperature (°C)	Carbon number
Gas	Below 20 °C	1-4
Petroleum ether	20-60 °C	5-6
Ligroin (light naphtha)	60-100 °C	6-7
Natural gasoline	40-205 °C	5-10 & cycloalkanes
Kerosene	175-325 °C	12-18 & aromatics
Gas oil	Above 275 °C	12 and higher
Lubricating oil	Non-volatile liquids	Long chains and cyclic structures
Asphalt	Non-volatile solids	Polycyclic structures

A.3.1.7 Surfactants

A surfactant combines in a single molecule a strongly hydrophobic group with a strongly hydrophilic group. They tend to congregate at the interfaces between the aqueous medium and the other phases of the system such as air, oily liquids and particles, thus imparting

properties such as foaming, emulsification and particle suspension [Standard Methods, 1998].

The hydrophobic group is generally a hydrocarbon containing about 10 to 20 carbon atoms. Hydrophilic groups are of two types, those that ionise in water (positively or negatively charged) and those that do not [Standard Methods, 1998].

A.3.1.8 Pharmaceuticals

Pharmaceutical compounds comprise drugs and medicinal chemicals used for both humans and animals. Some are isolated from natural sources (plant, animal or mineral). Many are synthesised in industrial processes for reasons of economy, purity and adequacy of supply. Although such chemicals are developed for therapeutic (*i.e.* beneficial) reasons for specific organisms, they can also be toxic.

Pharmaceuticals can enter natural waters through sewage effluent and landfill leachates and present an unknown risk to aquatic species [Pascoe *et al.*, 2003].

Some examples of pharmaceuticals are the following: antibiotics, hormones, pain killers, steroids.

Besides the final products themselves, the pharmaceutical manufacturing industry uses a wide variety of organic chemicals that can occur in their wastewaters, and hence potentially enter natural waters. The US EPA has published effluent limitations guidelines that list many specific organic compounds [US EPA, 1998]. They include the following:

- Alcohols (amyl alcohol, ethanol, isopropanol, methanol)
- Aldehydes (isobutyraldehyde)
- Alkanes (n-heptane, n-hexane)
- Amines (diethylamine, triethylamine)
- Aromatics (benzene, toluene, xylenes, chlorobenzene, o-dichlorobenzene)
- Chlorinated alkanes (chloroform, methylene chloride, 1,2-dichloroethane)
- Esters (ethyl acetate, isopropyl acetate, n-amyl acetate, etc.)
- Ethers (tetrahydrofuran, isopropylether)
- Ketones (acetone, 4-methyl-2-pentanone)

A.3.1.9 Naturally occurring toxicants

Many compounds that can exhibit toxicity occur naturally in the environment. These include many heavy metals, other inorganic substances and various organic compounds. The inorganic substances (including heavy metals) appear in the natural environment through contact between natural waters and the local geology. They simply dissolve, or are weathered, out of the local rocks and soils.

A particularly important class of organic toxicants is the cyanotoxins. They are released into the water when the cells of cyanobacteria (also called blue-green algae) are ruptured (*e.g.* by decay or algicides). Passive release can also occur [DWAF 2002b]. The appearance of cyanobacteria is one symptom of eutrophication of natural waters caused by increasing nutrient loads. Some degree of monitoring for cyanotoxins is carried out in the National Eutrophication Monitoring Programme [DWAF, 2002b].

A.3.2 Factors affecting their extent

The extent to which a toxicant occurs in the aquatic environment is represented by its concentration. This concentration depends, on the one hand, on the nature of the toxicant and various properties of it and, on the other, various properties of the aquatic environment in which it finds itself. Some of these properties are illustrated in Figure A.7.

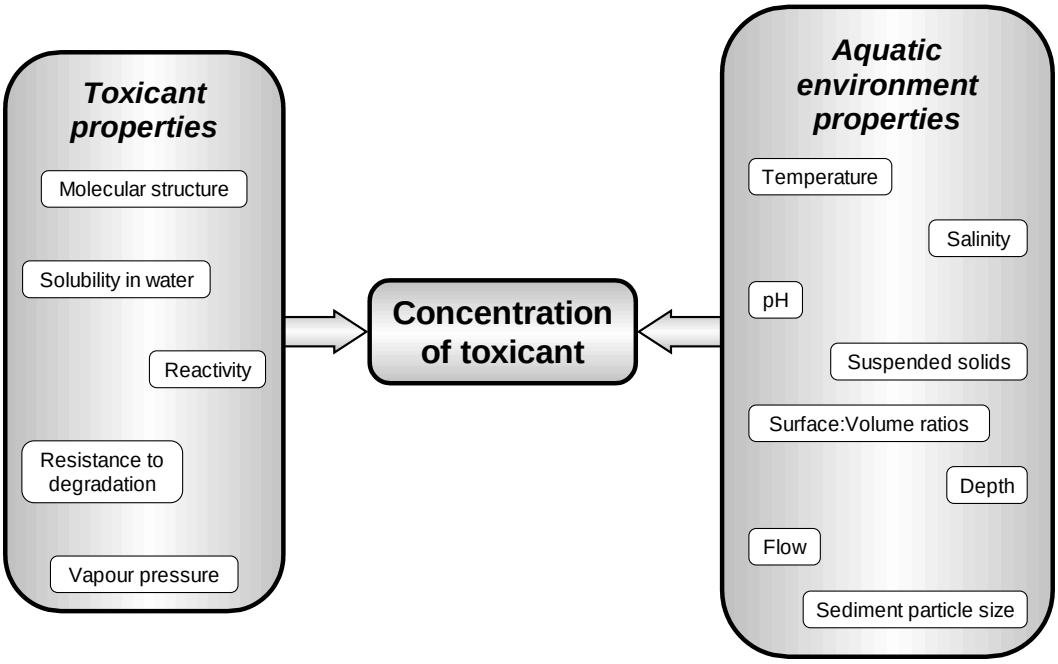


Figure A.7. Properties of the toxicant and aquatic environment that determine the concentration of the toxicant.

A.3.3 Measurement of their extent

The analytical measurement of the concentration of toxicants depends primarily on the nature of (i) the toxicant and (ii) the medium in which it occurs. In the current context, three media are possible, namely water, sediment and biological organisms (like fish).

Water is the most common non-gaseous medium in which toxicants occur and is the medium for which most analytical techniques have been developed. The concentration of the toxicant is a well-defined quantity expressed as the amount (usually as mg or µg) per unit volume (usually litre).

In respect of sediments and organisms, toxicants are often thought of as "accumulating" in these media. Sediments can act as "sinks" in which toxicants can gradually increase in concentration over time, even though the concentration in the bulk water (with which it is in contact) might remain fairly constant. Nevertheless, even sediments have an upper limit of solubility. As long as the environmental conditions (both chemical and physical) do not change, these higher concentrations in sediments can remain stable and the toxicant is effectively trapped. However, these situations are sometimes referred to "time bombs" because there is often no guarantee that the conditions will indeed remain constant indefinitely. For example, flooding can scour out sediments, mixing them with the bulk water and thus potentially "releasing" the toxicants into the water and transporting them downstream.

The toxicant typically needs to be extracted from the sediment or organism into a liquid phase (water or an organic solvent). This liquid phase is then subjected to analytical measurement.

Accumulation of toxicants in the organs and tissues of biological organisms is referred to as "bioaccumulation". For example, some fish accumulate pesticides and heavy metals.

Various reference methods for some toxicants are suggested in the South African Water Quality Guidelines [DWAF, 1996a-g]. For more detail these guidelines should be consulted. For details on some individual analytical methods, the latest Standard Methods (1998) can be consulted.

A.3.3.1 Inorganic substances

Analytical methods for heavy metals and inorganic compounds, including sample preservation methods, are well known, standard and widely practised [Standard Methods, 1998]. The most common analytical methods for heavy metals use Atomic Adsorption Spectrometry (AAS) and the Inductively Coupled Plasma (ICP) technique. The other inorganic substances usually make use of classical "wet chemical" techniques and automated variations of them as well as more modern instrumental techniques like ion chromatography [Standard Methods, 1998].

In most cases, the total concentration of a heavy metal or other inorganic substance will be measured and little or no attention given to the forms in which it exists (*i.e.* its "speciation"). However, filtering of samples can separate solid forms from dissolved forms, allowing this distinction to be made relatively easily.

A.3.3.2 Pesticides

Standard techniques are available for organochlorine pesticides and chlorinated phenoxy acid herbicides [Standard Methods, 1998]. Local capacity exists (*e.g.* at the ARC) using a range of chromatographic methods capable of determining a wide range of individual pesticides as well as classes of pesticides.

A.3.3.3 Petroleum products

At present some standard procedures are available for determining "oil and grease" and hydrocarbons [Standard Methods, 1998]. Local capacity also exists (*e.g.* at the SABS) for analysing for "total petroleum hydrocarbons", "diesel range organics" (10-28 carbons) and "gasoline range organics" (6-10 carbons).

A.3.3.4 Pharmaceuticals

Regarding many of the organic chemicals used in the pharmaceutical manufacturing industry, the US EPA has published guidance on appropriate analytical methods [US EPA, 1999]. Procedures that can be used include biological (using *Hydra vulgaris*) [Pascoe *et al.,* 2003] spectrofluorometrical [Manzoori and Amjadi, 2003], spectrophotometrical and chromatographical [Schellen *et al.,* 2003].

A.3.3.5 Surfactants

Standard techniques are currently available that determine surfactants [Standard Methods, 1998].

A.3.3.6 Bioaccumulation analyses

Bioaccumulation analyses are used to determine the degree to which a toxicant accumulates in an organism when subjected to long-term exposure [Rand and Petrocelli, 1995]. A bioconcentration factor can be determined that reflects the ratio between average concentration of the toxicant in the tissues of the organism to the average concentration in the water to which they are exposed.