AQUATIC TOXICITY TESTING IN SOUTH AFRICA: STATUS OF AQUATIC TOXICITY TESTING IN SOUTH AFRICA

Report to the Water Research Commission

by

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AQUATIC TOXICITY TESTING IN SOUTH AFRICA: GUIDELINE FOR THE ACCREDITATION OF ROUTINE AQUATIC TOXICITY TESTING LABORATORIES (including a DVD with individual quality management documents)
WRC Report No. TT 504/11

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EXECUTIVE SUMMARY

The aims of this project were:

- To compile a quality assurance manual to guide South African aquatic toxicity testing laboratories;
- To develop an implementation plan for DWAF for routine toxicity testing; and
- To develop a guideline to promote a sustainable network between toxicity testing laboratories.

The results of this project are published in two reports:

- Volume 1: STATUS OF AQUATIC TOXICITY TESTING IN SOUTH AFRICA (this report)
- Volume 2: GUIDELINE FOR THE ACCREDITATION OF ROUTINE AQUATIC TOXICITY TESTING LABORATORIES. This also contains a DVD with about 200 individual quality management documents that can be customised to individual manager's requirements.

The specific aims of the work involved in producing Volume 1 were the following:

- Determine the role aquatic toxicity testing plays in the protection of water resources internationally.
- Determine the status quo of aquatic toxicity testing in South Africa.
- Formulate a draft implementation plan for routine aquatic toxicity testing in South Africa addressing management and operational structures, and capacity building.

Standardised toxicity test protocols and the application of these tests in the assessment of complex industrial wastes have led to routine toxicity testing in many countries (e.g. Germany, Canada, United States of America, and New Zealand) for compliance and regulatory monitoring. Toxicity limits are set by the regulatory authority and are used in the same manner that chemical limits are used.

The toxicity of toxins and toxicants is described in terms of the dose that causes a particular effect in a specified population. The concentration is expressed as the Effective Concentration (EC) or Lethal Concentration (LC) that elicits a response from 50% of the exposed population (EC $_{50}$ or LC $_{50}$). Toxicity Units (TUs) are used to describe concentration-based toxicity measurements and are typically included in wastewater discharge permits. The application of a safety factor to measured TUs provides a reliable estimate of effluent concentrations that are safe for most ecologically important aquatic species.

As far as the status quo of toxicity testing in South Africa is concerned, discussions were held with representatives from the Department of Water Affairs (DWA) including the national department responsible for implementation of routine toxicity testing and the regional departments responsible for monitoring. It was concluded that they were not sufficiently up to standard with respect to the management and technical components of routine aquatic toxicity testing. Steps need to be taken to address this situation and to address the potential loss of critical expertise when key staff members leave. At present toxicity testing is requested by DWA on an *ad hoc* basis.

A survey conducted amongst aquatic toxicity testing laboratories and consultants revealed that, should routine aquatic toxicity testing be implemented, the provision of toxicity testing services will be constrained by a lack of suitable analytical capacity. Furthermore, most of the current laboratories are located in Gauteng

while only four laboratories are SANAS (South African National Accreditation System) accredited facilities. Current turnover of samples in most instances is too low to make running an accredited facility a viable option. The absence of standardised methods and toxicity test standards are detrimental to service quality and the interpretation of results – a prerequisite for the provision of reliable, legally defensible results to the client. Client understanding of toxicity testing and the application of results need to be improved. Greater participation in policy formulation and interaction between stakeholders are also required.

The following specific findings were recorded at a workshop attended by representatives from aquatic toxicity testing laboratories:

- 18 of the 27 laboratories and individuals involved with toxicity testing are located in Gauteng.
- Representatives are aware of Direct Estimation of Ecological Effect Potential (DEEEP) but are disappointed regarding its current application in South Africa.
- 20 to 50 samples per month would make providing a toxicity testing service and associated capacity building a viable option.
- Accreditation is too costly under current circumstances.
- Funding and training assistance to establish operational units would enhance service delivery on a national scale.
- Outside Gauteng there are no toxicity testing facilities in close proximity to industries discharging effluents into water resources.

Conclusions

- The successful implementation of routine toxicity testing nationwide will depend on the interactive collaboration between DWA, toxicity test service providers, industry and the general public. Structures to encourage this collaboration will thus have to be put in place.
- Toxicity testing laboratories need a legislative incentive, e.g. implementation of the DEEEP approach, to invest in capacity building (human resources and accreditation of test methods).
- DWA personnel will have to be trained to include ecotoxicity guidelines and ecotoxicity testing in licensing conditions for effluent discharge and to enforce compliance with these conditions.
- Communication between the personnel driving the implementation of the DEEEP approach at
 national level and the personnel enforcing the licensing conditions will be of the utmost importance to
 the success of the implementation strategy and to allow for timeous interventions.
- A communication strategy that involves the general public, laboratories, municipalities and relevant government departments will aid in raising the profile of water quality testing.
- In case of the full national implementation of routine toxicity testing, a shortage of capacity (i.e. a competent human resource component and accredited laboratories) to provide these services will impact negatively on the implementation process. To sustain and improve the quality of aquatic toxicity testing in South Africa and assist in the implementation of routine toxicity testing as required by the DEEEP and the NTMP, DWA will have to implement their proposed Laboratory Strategy. This will require laboratories undertaking water quality testing on behalf of municipalities to have every individual method used evaluated and approved if the results are to be accepted by the DWA.
- Quality guidelines for toxicity testing laboratories covering test methods, analyses of test results, infrastructure and training of personnel are thus required. The toxicity testing laboratories should therefore also be assessed for compliance with the guidelines, e.g. through accreditation by SANAS.
- The need for affordable human capacity building can be addressed by in-service training for graduates and regional training courses. A central analytical laboratory, e.g. the Resource Quality Services (RQS) laboratory in conjunction with the accreditation body SANAS can act as an information hub for laboratories that will participate in toxicity testing for regulatory purposes.

 Networking between laboratories that have already achieved accreditation for the purpose of toxicity testing (including the RQS laboratory) and those laboratories seeking accreditation status will foster a culture of cooperation between laboratories in terms of training, monitoring the quality of analyses and providing a cost-effective service in order to achieve national objectives.

Recommendations

- To implement internationally accepted aquatic toxicity tests as part of the South African regulatory process, it is suggested that the following actions be taken:
 - An in-depth analysis and classification of the numerous toxicity test procedures and methods to determine their suitability to application in the various fields of water use and discharge.
 - > The incorporation of the above toxicity tests in the formulation of environmental legislation and policy directives (i.e. licensing conditions requiring ecotoxicity measurements must be imposed).
 - The determination and implementation of compliance limits based on chemical and toxicity guidelines to ensure efficient monitoring and enforcement.
- It is proposed that a reference framework similar to the framework adopted by the United Nations GEMS/Water Programme be developed that includes the following.
 - ➤ A Methods Manual containing validated, standardised toxicity tests for regulatory compliance.
 - Laboratory performance evaluation by compulsory participation in proficiency testing schemes and follow-up training if performance is not up to standard.
 - Establishment of a National Toxicity Testing Laboratory Accreditation Programme (NTTLAP).
- It is proposed that the following steps be taken to facilitate the national Implementation of routine aquatic toxicity testing.
 - The appointment of a National Coordinating Manager by the national department (DWA) to ensure the successful implementation of routine toxicity testing and to serve as a central point of communication for industries and DWA regions as well as representatives from laboratories.
 - ➤ Regional champions, appointed by the National Coordinating Manager, must ensure that the regional offices in Water Management Areas (WMAs) have the capacity to implement toxicity testing routinely. The regional champions will require the support of the Catchment Management Agencies (CMAs) to ensure successful implementation of routine aquatic toxicity testing in a specific region. As was recommended in the NTMP, it is important that toxicity monitoring and the requirements for toxicity testing be implemented in the WMA through the catchment management strategy of the CMA.
 - It is recommended that implementation of routine toxicity testing in the various regions of South Africa be phased in region by region. Regional champions should be engaged in the process unless other persons are identified by the national coordinating manager. In addition, all existing licence/permit holders should be involved in the implementation process.
 - Ongoing consultation with affected catchment and discharger/polluter representatives during the implementation phase is recommended to ensure that the concerns of all stakeholders are addressed. Successful water resource management is dependent on cooperation among representatives of all spheres of government and the active involvement of water users and other organisations and interested and affected stakeholders.
 - > Expectations of interested and affected stakeholders with respect to legal action for non-compliance should be gauged. Trained enforcement officers are required to identify dischargers not complying with their licence conditions.

- ➤ Feedback to the National DWA Coordinating Manager from all regions of South Africa as well as industry is required to address all problems and successfully manage a nationwide process.
- It should be guaranteed that monitoring information is available at written request as stipulated by the Promotion of Access to Information Act. This applies to information contained in any national monitoring system established in terms of the Water Act.
- > Specific guidelines for the processing and storage of data in the public domain must be available.
- Industries must be made aware of the need for toxicity testing and informed of the facilities where such testing can take place.
- It is recommended that toxicity testing facilities operate within regional laboratory networks supported by DWA and the South African National Accreditation System (SANAS).
 - The need for technical assistance is foreseen with regards to the setting-up of analytical facilities and facilities seeking accreditation in terms of regulatory requirements. Networks between laboratories that have already achieved accreditation for the purpose of toxicity testing and those laboratories seeking accreditation status should be set up to foster a culture of cooperation to achieve national objectives.
 - > Capacity in terms of testing facilities and human resources must be increased. Existing facilities need a regulatory incentive from government to maintain existing capacity.
 - ➤ The need for affordable human capacity building can be addressed by in-service training for graduates and regional training courses. A core analytical laboratory, e.g. the Resource Quality Services (RQS) laboratory in conjunction with the accreditation body SANAS, can act as an information hub for laboratories that will participate in toxicity testing for regulatory purposes.
- It is also recommended that the following topics be addressed in future WRC projects.
 - ➤ The practical training of staff employed by toxicity testing laboratories.
 - > The revision of aquatic chemical and toxicity guidelines. These guidelines should also address risk determination, i.e. the probability that a chronic effect on a test organism does not exceed a certain level.
 - The development of a chronic toxicity test for water (falling in the ecological category "Fair and Good") testing.

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LIST OF ABBREVIATIONS

ANZECC	Australian and New Zealand Environment Conservation Council
ASTM	American Society for Testing and Materials
CAEAL	Canadian Association of Environmental Analytical Laboratories
CEPA	Canadian Environmental Protection Act
CMA	Catchment Management Agency
CSIR	Council for Scientific and Industrial Research
CWA	Clean Water Act
DEAT	Department of Environmental Affairs and Tourism
DEEEP	Direct Estimation of Ecological Effect Potential
DG	Director General
DTA	Direct Toxicity Assessment
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
EC	Environment Canada
EC	Effective Concentration
EC ₅₀	Median effective concentration
EEC	European Economic Community
EPA	Environmental Protection Agency
EPS	Environmental Protection Series
EU	European Union
GEMS	Global Environmental Monitoring System
GLP	Good Laboratory Practice
IEC	International Electrotechnical Committee
INELA	Institute for National Environmental Laboratory Accreditation
ISO	International Organisation for Standardisation
LC	Lethal Concentration
LC50	Median lethal concentration
NELAC	National Environmental Laboratory Accreditation Conference
NGO	Non-Governmental Organisation
NLA	· · · · · · · · · · · · · · · · · · ·
NOEC	No Observed Effect Concentration
NPDES	National Pollutant Discharge Elimination System
NTMP	National Toxicity Monitoring Programme
NTTLAP	National Toxicity Testing Laboratory Accreditation Programme
NWA	National Water Act
NWI	National Water Initiative
NWP	,
NWQMS	National Water Quality Monitoring Strategy
OECD	Organisation for Economic Cooperation and Development
PAIA	Promotion of Access to Information Act
PTS	Proficiency Testing Scheme
QA	Quality Assurance
QC	Quality Control
RC	Regional Committee
RDM	Resource-Directed Measures
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals

RHP River Health Programme

RIZA Netherlands' Institute for Inland Water Management and Waste Water Treatment

RLN Regional Laboratory Network

RM Reference Method

RQS National Resource Quality Services

SALGA South African Local Government Association

SANAS South African National Accreditation System

SANS South African National Standard

SDC Source-Directed Controls

TNI The NELAC Institute

TU Toxicity Unit

UK United Kingdom

USA United States of America

WET Whole Effluent Toxicity

WETT Whole Effluent Toxicity Tests

WFD Water Framework Directive

WMA Water Management Area

WMS Water Management System

WRC Water Research Commission

TERMINOLOGY

Accreditation. A formal procedure to determine the competence for performing certain kinds of measurements by taking into account set criteria.

Acute. Refers to a stimulus that rapidly (within 96 hours) induces a lethal or sub-lethal effect.

Ad hoc testing. Once-off non-routine testing.

Aquatic toxicity testing. A technique used to determine the effect of a series of concentrations of a chemical, water or effluent on a group of organisms under controlled laboratory conditions. Effect is measured in terms of lethality (number of organisms affected) or the degree of effect, e.g. percentage growth inhibition.

Chemical. Any element, compound, formulation, or mixture of chemical substances that might enter the aquatic environment through spillage, application, or discharge. Examples of chemicals that are applied to the environment are insecticides, herbicides and fungicides.

Chronic effect. An adverse effect on any living organism in which symptoms develop slowly over a long period of time or recur frequently. Such effects may be related to changes in metabolism, growth, reproduction or ability to survive.

Control sample. A control sample duplicates all exposure conditions, but contains no toxicant. The control is used to determine the absence of measurable toxicity due to basic test conditions.

Definitive test. Toxicity test designed to establish the concentration at which a particular end point occurs. Exposure for these tests incorporates multiple concentrations at close intervals and multiple replicates. A range comprising at least five concentrations/dilutions where, ideally, the highest concentration will result in a 100% inhibition and the lowest concentration in 0% inhibition, e.g. 100%, 50%, 25%, 12.5% and 6.25%.

 \textit{EC}_{50} value. The median effective concentration, i.e. the concentration of a toxicant in water (e.g. mg/ ℓ) that is estimated to cause a discernible sub-lethal toxic effect to 50% of the test organisms. In most instances the EC $_{50}$ value and its 95% confidence limits are statistically derived by analysis of an observed response for various test concentrations after a fixed period of exposure. The duration of exposure must be specified (e.g. 72 hours).

Effective concentration (EC). The EC is the point estimate of the toxicant concentration at which a certain percentage of the test organisms would be affected, such as bacterial growth inhibition (e.g. EC_{20} being the test concentration causing 20% inhibition or EC_{50} the concentration causing 50% inhibition).

Effluent. Any liquid waste (e.g. industrial, municipal) discharged into the aquatic environment. These effluents, treated or untreated are considered to be complex when they contain 10% or more by volume of industrial wastewater (defined in Regulation 1191 of 8 October 1999 under the National Water Act of 1998).

 IC_{50} value. Median inhibition concentration, i.e. the concentration estimated to cause a 50% reduction in growth rate in an organism, compared to a control. The exposure time must be specified, e.g. "IC₅₀ (72 hours)" for growth rate derived IC₅₀ values and a test duration of 72 hours.

Inter-laboratory study. Test result comparison between two or more laboratories testing the same sample using the same or different methods.

Intra-laboratory trials. Same sample tested by three or more technical personnel and the results statistically analysed for acceptability.

ISO standards. Formal agreements between ISO's members on content. These standards are developed by technical committees representing many countries.

LC₅₀ **value**. The median lethal concentration, i.e. that concentration of the test substance in water which kills 50% of a test batch of test organisms (e.g. Daphnia or fish) within a particular period of exposure, which must be stated.

Leachate. Water or wastewater that has percolated through a column of soil or solid waste within the environment.

Licence. A legal document that entitles a person to use water within the terms and conditions of the licence (also referred to as a water use authorisation). These terms and conditions might be subject to review after a period listed in the licence, which may be any period not exceeding 5 years.

Lowest-observed-effect concentration (LOEC).

This represents the lowest concentration of a test material or substance to which organisms were exposed and for which a statistically significant effect was observed relative to the control.

Management. All the activities that are used to coordinate, direct and control an organisation.

Monitoring. The routine (e.g. daily, weekly, monthly, and quarterly) checking of quality or collection and reporting of information. It refers either to the periodic (routine) checking or measurement of certain biological or water-quality variables, or the collection and testing of samples of effluent, elutriate, leachate or receiving waters for toxicity.

No-observed-effect concentration (NOEC). This represents the highest concentration of a test material or substance to which organisms are exposed and in which no significant change in effect is apparent relative to the control.

Parameters. A set of measurable factors with limits that define a system and control its behaviour.

Quality. The totality of an entity's properties which make it capable of satisfying an expressed or hypothetical need, that is, acceptability or suitability for a given purpose.

Receiving water. Surface water (e.g. stream, river, or lake) that has received a discharged waste or else

is about to receive such a waste, e.g. just upstream from the discharge point.

Reference toxicant. A standard chemical used to measure the sensitivity of the test organisms to establish confidence in toxicity data obtained for a test material or substance. In most instances a toxicity test with a reference toxicant is performed to assess the sensitivity of the organisms at the time the test material or substance is evaluated and the precision of results obtained by the laboratory for that chemical.

Sample. The undiluted product or effluent received for testing.

Sampling. A defined procedure involving a part of a substance, material or product taken to provide for testing or calibration. It is typically a representative sample of the whole. Sampling may also be required by the appropriate specification for which the substance, material or product is to be tested or calibrated.

Screening test. An initial screening where 100% of an effluent and or receiving water sample or 100 mg/ ℓ of a product/chemical is used to determine if the sample is toxic or not.

Semi-static test. A test without flow of solution, but with occasional batch-wise renewal of the test solution after prolonged periods (e.g. 24 hours).

standard Operating Procedure. Complete reference document or operations manual that provides the purpose, authorities, duration and details of the preferred method of performing a single function or a number of interrelated functions in a uniform manner. In case of a toxicity test it is step-by-step instruction on how to do a particular test, including specimen requirements, environmental conditions, reference ranges, and reporting units to ensure that operations are carried out correctly and always in the same manner.

Sub-lethal. Detrimental to the organism but below the level that directly causes death within the test period.

Technical. Relating to a practical subject organised on scientific principles.

Test sample. The aqueous sample that is to be tested. It may be derived from chemical stock solutions or collected from effluents, elutriates, leachates, or receiving waters.

Top management. Person or group of people at the highest level within an organisation. They coordinate, direct and control organisations.

Toxicity. The inherent potential or capacity of a chemical to cause adverse effects on living organisms.

Toxicity test. A method to determine the effect of a material on a group of selected organisms under defined conditions. An aquatic toxicity test usually measures either (a) the proportions of organisms affected (quantal), or (b) the degree of effect shown (graded or quantitative) after exposure to specific concentrations of chemical, effluent, elutriate, leachate, or receiving water.

Toxicity unit (acute) (TUa). The concentration of the undiluted effluent (i.e.100%) divided by the concentration of the effluent that caused 50% lethality (LC₅₀) of the test organisms at the end of the acute exposure period.

Toxicity unit (chronic) (TUc). The concentration of the undiluted effluent (i.e.100%) divided by the concentration of the effluent that caused no-observed-effect on the test organisms at the end of the chronic exposure period.

Upstream water. Surface water (e.g., in a stream, river, or lake) that is not influenced by the test material or substance by virtue of being removed from it in a direction against the current or sufficiently far across the current.

Validation. Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled and the ability of the laboratory to achieve satisfactory performance against documented performance characteristics.

Verification. In-house checks to verify that the equipment is still accurate using a reference traceable to national standards.

Wastewater. A general term that includes effluents, leachates, and elutriates.

Water resources. A term used in the NWA (Chapter 1.(1)) to refer to watercourses, surface waters, estuaries, or aquifers. The term covers the water itself, sediments, and associated biota (e.g. plants, animals and micro-organisms).

CHAPTER 1: INTRODUCTION

1.1 EXISTING PROGRAMMES

The South African National Water Act (Act 36 of 1998) (NWA) mandated the establishment of national monitoring systems to reduce and prevent degradation of water resources and to assess their quality. South Africa's water resources comprise inland surface water, water courses (rivers, springs, natural channels, wetlands, lakes and dams into which and from which water flows), estuaries and aquifers. A crucial implication of the Act is that an ecological effect-based approach needs to be applied to water resource management (DWAF, 2003) thus supporting regular toxicity testing of water resources as well as complex industrial wastewaters (effluents) which are released into water resources.

To comply with these requirements of the NWA, the National Toxicity Monitoring Programme (NTMP) (DWAF, 2005) for water resource management and the Direct Estimation of Ecological Effect Potential (DEEEP) (DWAF, 2003) approach to manage effluent discharge into surface waters were designed. The first step towards implementation of the DEEEP approach was the compilation of a "Methods Document" addressing the selected ecological hazard parameters and tests to assess these parameters (Slabbert, 2004). This methods document describes the key practical steps involved in the execution of these tests. Quality requirements pertaining to standard operating procedures for the practical execution steps as well as training requirements for new staff and management requirements for the toxicity testing laboratory to comply with minimum requirements for accreditation purposes according to the International Standard ISO/IEC 17025 (ISO 17025:2005), were not addressed.

The DEEEP approach for wastewater monitoring has been implemented since 2005 as a pilot study. Some of the anticipated key success factors in the implementation of the DEEEP approach were the following:

- Development of a mechanism for networking, coordination and capacity building.
- Collaboration between different public and private sector stakeholders and training institutions in terms of financial support, manpower requirements, capacity building and logistical support in fulfilment of the requirements of the NWA (DWAF, 2003).

Although not documented, it could be expected that the same success factors would apply to the successful implementation of the NTMP. The current work was intended to promote the full scale implementation of the DEEEP approach and the NTMP.

1.2 MANAGEMENT OF WATER RESOURCES

Aquatic toxicity testing is seen as an integrator instrument to indicate the effects caused by the combined toxicity of all chemicals contained in a water sample. It is a relatively simple laboratory procedure and employs the use of standardised surrogate plants, invertebrates, bacteria and vertebrates to measure the total integrated toxic effect of, for example, a complex effluent directly (EPA, 1991). These whole effluent toxicity tests (WETT) are applied at end-of-pipe before the effluent is discharged into the receiving water allowing the effects of the effluent to be isolated from any in-stream effects. The advantages and disadvantages of the WETT approach are listed in **Table 1.1**.

Table 1.1. Advantages and disadvantages of the whole effluent toxicity testing approach (adapted from Hall and Golding, 1998; EPA, 1991)

Advantages	Disadvantages				
Aggregate toxicity of all constituents in a complex effluent is measured	Properties of specific chemicals are not assessed				
Unknown toxicants are addressed	There is no identification of specific toxic components				
The bioavailability of the toxic constituents is assessed and the effects of interactions between constituents are measured	No persistency or sediment coverage				
Site-specific toxicology	Incomplete toxicology due to few species being tested				
The concept is easily understood and provides tangible evidence of environmental impact or lack thereof	Toxicity of contaminants where there are chemical/physical conditions present that act on toxicants in such a way as to "release" toxicity downstream will not be measured				

The availability of standardised toxicity test protocols for WETT and the application of these tests in the assessment of complex industrial wastes have led to routine toxicity testing in many countries (e.g. Germany, Canada, the United States of America (USA) and New Zealand) for compliance and regulatory monitoring. Toxicity limits are set by the regulatory authority and have been included internationally (e.g. USA and Canada) as conditions of effluent discharge licences. Toxicity limits are used in the same manner that chemical limits are used and should be viewed as equivalent to emission standards for chemical and physical parameters.

It is important to note however, that toxicity tests can only measure the toxicological properties they are designed to detect (EPA, 1991). **Figure 1.1** illustrates the management framework that underpins toxicity testing as a tool to protect water resources.

Testing and data reporting are the responsibility of the testing laboratory. Sampling is more often than not done by the laboratory. The design of the sampling regime is a cooperative effort between the policy maker, the scientist/technician who is an expert in the limitations and practical application of the test and the enforcer/manager who uses the outcome to craft catchment characteristics to accomplish the vision for the catchment/receiving water. The cost associated with performing the analyses on the effluent however is the responsibility of the discharger.

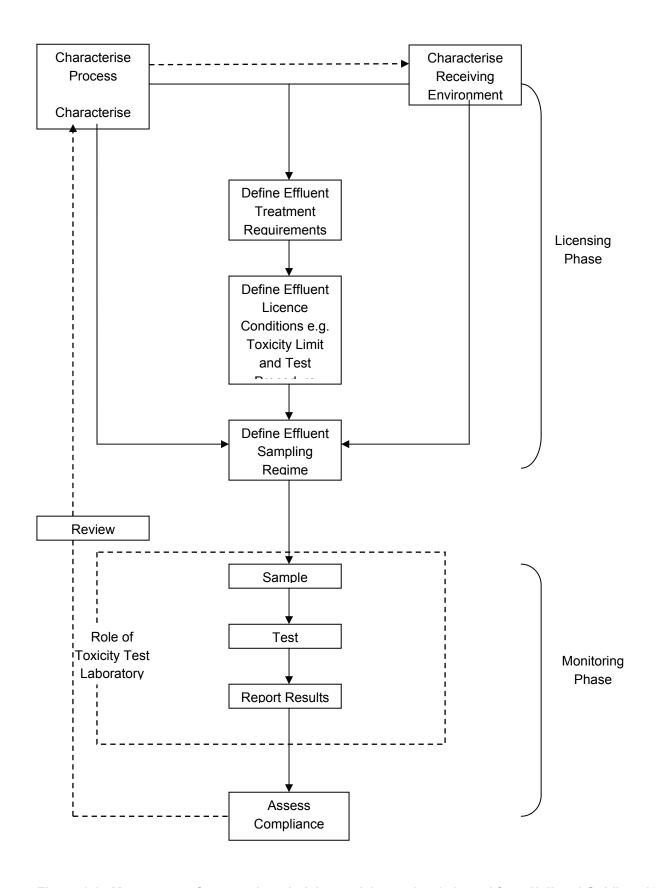


Figure 1.1. Management framework underlying toxicity testing (adapted from Hall and Golding, 1998)

1.3 SETTING AND APPLYING TOXICITY LIMITS

The toxicity of toxins and toxicants are described in terms of the dose that causes a particular effect in a specified population. In other words, the concentration of exposure that causes a particular response. Typically, this concentration is expressed as the Effective Concentration (EC) or Lethal Concentration (LC) that elicits a response (e.g. immobility, lethality, etc.) from 50% of the exposed population (EC $_{50}$ or LC $_{50}$). Toxicity and ECs are inversely related – the lower the EC, the higher the toxicity (EPA, 1991). In order to minimise the confusion that is caused by the foregoing inverse relationship, Toxicity Units (TUs) are used to describe concentration-based toxicity measurements. These TUs are typically included in wastewater discharge permits. The number of toxic units in an effluent is described as 100 divided by the measured EC (where acute toxicity is being measured) expressed as a percentage:

$$TU_a = 100/LC_{50}$$

Where chronic toxicity is measured, the TU_c is determined by dividing 100 toxic units by the No Effect Concentration (NOEC).

Uncertainties related to the sensitivity of individual organisms and the unknown group sensitivity of organisms are not represented in toxicity tests. One of the fundamental principles of toxicology is that each individual in a population responds to stress in an individual manner (Boelens, 1980). The application of a safety factor to measured TUs is one way of reducing uncertainties related to unknown individual and group sensitivities. This provides a reliable estimate of effluent concentrations that are safe for most ecologically important aquatic species.

However, the magnitude of safety factor for application is widely debated in literature. Proposals vary from 1/10 to 1/100 but general consensus remains that it seems logical that a safety factor should be linked to effluent toxicity and be applied nationally. The chosen factor could be either built into the toxicity limit or be included in the form of a minimum dilution required (Boelens, 1980).

1.4 IN SUPPORT OF THE SOUTH AFRICAN NATIONAL WATER ACT

The South African National Water Act (Act 36 of 1998) (NWA) mandates the establishment of policies and approaches to reduce and prevent degradation and to assess the quality of water resources. To comply with these requirements, the Direct Estimation of Ecological Effect Potential (DEEEP) approach for complex industrial wastewater discharge (DWAF, 2003) and the National Toxicity Monitoring Programme for Surface Waters (NTMP) (DWAF, 2005) were introduced by the Department of Water Affairs and Forestry (DWAF) to monitor discharges into surface water.

The DEEP approach aims to assess the ecological hazard posed by complex industrial wastewater discharges. In recent years it has become increasingly apparent that substance-specific methods and chemical data alone were not effective in assessing the direct environmental toxicity hazard of discharges containing mixtures of substances (DWAF, 2003). The proposed methodology will make use of both standard acute and chronic tests to set limits using wastewater toxicity as the control parameter.

The NTMP is essentially a "status and trends" monitoring programme of water quality relating to toxicants and toxicity (DWAF, 2005). It aims to report on both the potential for toxic effects to selected test organisms and on potentially toxic substances in South African inland surface water resources (DWAF, 2005). The NTMP was designed in anticipation of the DWAF's Resource Classification System (Murray et al., 2004). The NTMP will play a support role and provide supplementary information to monitoring programmes that will focus on determining resource quality objectives for South African water resources. As such, the NTMP has a potentially important role to play in water resource management.

At present the DEEEP method is in a pilot implementation phase and in order to promote the full scale implementation of the DEEEP approach and the NTMP in support of the NWA, an implementation plan for routine toxicity testing by the DWA is required.

CHAPTER 2: INTERNATIONAL AND NATIONAL LEGISLATIVE AND POLICY FRAMEWORK

2.1 INTERNATIONAL LEVEL

2.1.1 United States of America

The Clean Water Act (CWA) states that "It is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited" (EPA, 1991). The CWA and Environmental Protection Agency (EPA) regulations authorise the use of three control approaches to achieve and maintain water quality standards. These are:

- Chemical Specific Approach (chemical-specific water quality based limits that include an acute and chronic value).
- Whole Effluent Treatment (WET) control approach (acute and chronic toxicity tests are used to measure toxicity of wastewaters to aquatic organisms).
- Biological Bioassessment approach (incorporates chemical, physical and biological data).

The National Pollutant Discharge Elimination System (NPDES) Permit Program controls water pollution by regulating point sources that discharge pollutants into waters of the USA. The toxicity tests that NPDES permit holders are required to conduct are standardised test methods (e.g. Acute Daphnia, Fish and Algal tests) published by the EPA in which effluent concentration is the primary variable by which the response is evaluated. In order for the toxicity tests to meet regulatory requirements participating laboratories need to employ good laboratory practices. These laboratories are certified by the EPA's Division of Water Quality to conduct the recommended toxicity tests for NPDES purposes (Division of Water Quality, 2001).

2.1.2 Canada

The Canadian Environmental Protection Act, 1999 (CEPA) was promulgated in March 2000 and its objective is to prevent pollution and protect the environment and human health. The act requires that toxicity testing for registration of new substances conform to Organisation for Economic Cooperation and Development (OECD) standards of Good Laboratory Practice (GLP) (Report EPS1/RM, 1996). In addition to the CEPA, the Canadian Fisheries Act (R.S., 1985, c. F-14) was established to protect Canada's fisheries resources and supporting habitats. It prohibits the deposition of substances in waters inhabited by fish that may be harmful to fish. Canada's Toxics Substances Management Policy, 1995 has two main objectives:

- The virtual elimination from the environment of toxicants that are persistent and bioaccumulative, and
- The management of other toxic substances to prevent or minimise their release into the environment (Environment Canada, 2004).

In order for the toxicity tests to meet regulatory requirements, the Canadian Inter-Governmental Aquatic Toxicity Group proposed the development and standardisation of a set of single species aquatic toxicity tests. Environment Canada has developed four Reference Toxicity Methods that are used to assess compliance. The test methods are:

 Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout (EPS1/RM/13, 2000)

- Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to Daphnia magna (EPS1/RM/14,2000)
- Biological Test Method: Reference Method for Determining Acute Lethality of Sediment to Marine or Estuarine Amphipods (EPS1/RM/35,1998)
- Biological Test Method: Reference Method for Determining the Toxicity of Sediment Using Luminescent Bacteria in a Solid-Phase Test (EPS1/RM/42,2002

Environment Canada defines a reference method as a specific biological test method for performing a toxicity test. It contains a set of explicit instructions and conditions which are described precisely in a written document. In contrast with other multi-purpose generic biological test methods published by Environment Canada, the use of a reference method is frequently restricted to testing requirements associated with specific regulations. Reference methods are favoured for use in:

- Governmental and provincial environmental toxicity laboratories for regulatory testing.
- Regulatory testing that is contracted out by Environment Canada or other agencies/industries.
- Government, provincial or municipal regulations or permits as a regulatory monitoring requirement.
- Where there is a need for the provision for very explicit instructions (EPS 1/RM/13, 2000).

Compliance is measured as a *Pass* or *Fail* against the results generated from the reference method.

2.1.3 European Union (EU)

The Water Framework Directive (WFD) was promulgated in 2000 (Griffiths, 2002) and aims to protect and enhance the quality of all inland and coastal waters within defined river basin districts in Europe by improving and integrating the way water bodies throughout Europe are managed. It is often described as the most progressive piece of European legislation (Griffiths, 2002). In order to assess toxic impacts of contamination in the aquatic environment, acute toxicity tests are employed.

Toxicity testing of environmental agents to protect human and aquatic health occurs in the European Union (EU) under Commission Directives that directs the risk assessment of new substances, existing substances, and biocidal products (NRC, 2006).

Commission Directive 93/67/EEC lays down the principles for assessment of risks to man and the environment of substances notified in accordance with The New Substances Risk Directive (Council Directive 67/548/EEC). Under Directive 93/67/EEC, risk assessment shall entail at least hazard identification and where appropriate, dose (concentration) – response (effect) assessment, exposure assessment and risk characterisation for both human health and the environment. The Seventh Amendment to Directive 67/548/EEC dealing with risk assessment of new chemical substances was adopted by the European Commission in 1993 and requires the following set of standardised toxicity tests to be conducted (Furlong, 1995):

- · Acute toxicity for fish.
- Acute toxicity for daphnia.
- Algal growth inhibition.
- Bacterial inhibition.

2.1.4 Australia and New Zealand

The Australian and New Zealand Environment and Conservation Council (ANZECC) developed a set of guidelines "The Australian and New Zealand Guidelines for Fresh and Marine Water Quality" in 2000 for

managing water quality in Australia and New Zealand. The ANZECC guidelines provide an authoritative reference for water quality management in New Zealand and Australia, particularly for toxic contaminants. Guideline values for many toxicants are listed in the guideline documents and are derived from standardised toxicity tests (Hart, 2001). The primary objective of the guidelines is:

"To provide an authoritative guide for setting water quality objectives required to sustain current, or likely future, environmental values [uses] for natural and semi-natural water resources in Australia and New Zealand" (Department of Environment and Conservation, 2006).

The following steps summarise the implementation of the ANZECC guidelines (Department of Environment and Conservation, 2006):

- Environmental values and human uses: These are determined by the community for their waterways.
- Water Quality Objectives: These represent the community's environmental values for waterways expressed for each catchment in the state.
- **Protection levels:** These are set for each waterway according to its condition: high conservation value, slightly to moderately disturbed, or highly disturbed.
- Waterway issues and level of risk: What are the issues or problems which might threaten the achievement of local environmental values? What level of risk do these issues pose for local environmental values?
- Indicators: Choose the right indicators for the issues or problems for local environmental values.
- Trigger values: Trigger values for each indicator used to assess the risk to an environmental value:
 Within the trigger value range
 Low risk to the environmental value
 Possible risk to the environmental value

In Australia, the guidelines form the central technical reference of the National Water Quality Management Strategy (NWQMS) which the federal and all state and territory governments in Australia have adopted for managing water quality. A series of guidelines have been published under the NWQMS and cover the following topics:

- Policies and processes to achieve water quality.
- Effluent and sewerage system management.
- Urban stormwater and recycled water.
- Fresh and marine water quality.
- Monitoring and reporting.
- Groundwater protection.
- Drinking water.

The NWQMS is part of the Council of Australian Governments' Water Reform Framework and is acknowledged in the National Water Initiative (NWI). The NWI, signed in 2004, is the blueprint for water reform in Australia. The NWI represents a shared commitment by governments in Australia to increase the efficiency of Australia's water use, leading to greater certainty for investment and productivity, for rural and urban communities, and for the environment.

New Zealand's Resource Management Act (1991) seeks to safeguard the "life-supporting capacity of air, water, soil, and ecosystems" through sustainable management of natural and physical resources (Hall and Golding, 1998). In order to meet this requirement a consent authority cannot grant a permit, or allow as a permitted activity, any discharge of wastewater which may have "any significant adverse effects on aquatic

life" (Resource Management Act, 1991). According to Golding and Hall (1998), it is therefore essential that the techniques used to assess and monitor the discharges are sufficiently sensitive to detect potential toxic impacts. The authors propose three key methods for assessing the potential toxicity of an effluent:

- Quantification of the chemical components.
- Quantification of the biological toxicity of the effluent.
- Biological monitoring.

Chemical testing involves detailed chemical analysis to characterise the wastewater and identify the contaminants. Reference is then made to the ANZECC Water Quality Guidelines which give an acceptable concentration for the contaminants identified. To provide a useful tool for use by resource managers and dischargers, standard WETT protocols are required for freshwater and marine algae, invertebrates and fish. In New Zealand, WETT has been used since the mid 1980s, generally using species imported from overseas or using New Zealand species with protocols developed for similar species overseas (Hall and Golding, 1998). At present, standardised protocols for use with indigenous New Zealand marine and freshwater, algal, invertebrate and fish species are being used. These include standardised protocols for the freshwater invertebrates Ceriodaphnia dubia (Water Flea), and Paracalliope fluviatilis (amphipod), freshwater fish, Gobiomorphus cotidianus (Common Bully) and the marine algae Dunaliella tertiolecta and the marine fish Rhombosolea plebeia (Sand Flounder). New Zealand also has standardised protocols for the freshwater algae Selenastrum capricornutum and the marine invertebrate Fellaster zelandiae (sand dollar) (Hall and Golding, 1998).

2.2 NATIONAL LEVEL

2.2.1 The National Water Act

The South African National Water Act (Act 36 of 1998) (NWA) mandates the establishment of policies and approaches to reduce and prevent degradation and to assess the quality of water resources. The DWA, as public trustee of South Africa's water resources in accordance with the National Water Act (Act 36 of 1998), has the mandate to manage water resources in a sustainable and equitable manner, for the benefit of all persons (DWAF, 2003).

The National Water Policy (NWP) (DWAF, 1997) has three fundamental objectives for managing South Africa's water resources:

- To achieve equitable access to water (includes equity of access to water services, to the use of water resources, and to the benefits from the use of water resources).
- To achieve sustainable use of water by making progressive adjustments to water use with the
 objective of striking a balance between water availability and legitimate water requirements, and by
 implementing measures to protect water resources.
- To achieve efficient and effective water use for optimum social and economic benefit.

Both the NWA and NWP support the management of the nation's water resources. The objective of managing the quantity, quality and reliability of the nation's water resources is to achieve optimum, long-term, environmentally sustainable social and economic benefit for society from their use. The overall intention of environmentally sustainable water use is to balance water use with the protection of the resource in such a way that the resources are not degraded beyond recovery (DWAF, 1997).

The implementation of resource-directed measures (RDMs) and source-directed controls (SDCs) in water quality management helps to enable resource protection. Resource-directed measures deal with setting goals and objectives for water resources in the environment while SDCs specify the criteria for controlling impacts. These are exemplified by licences which specify allowable resource-use such as water abstraction, effluent discharge, and land-uses which result in stream-flow reduction (Scherman, 2001).

The management of water quality through the use of RDMs and SDCs provides the rationale for employing aquatic toxicity tests to protect the water resource and its ecosystems. According to Jooste and Herbst (2004), the management of single substances in water is not enough to adequately assess the ecological and toxicity hazard that are posed by complex industrial wastewater discharges. There are several reasons for this inadequacy. These include toxicological, environmental and analytical considerations. From a toxicological perspective, the multiplicity of substances makes it difficult to estimate what the effect of the mixture would be. The DEEEP approach for complex industrial wastewater discharge (DWAF, 2003) represents a comprehensive approach to holistically assess the potential toxicity hazard of complex industrial wastewater discharges as a means to protecting the ecological integrity of aquatic ecosystems.

2.2.2 Direct Estimation of Ecological Effect Potential (DEEEP)

The DEEEP approach assesses the ecological hazard posed by complex industrial wastewater. It is an effect-based approach that will make use of both standard acute and chronic tests to set limits using wastewater toxicity as the control parameter. The approach aims to obtain a better insight into the effect of mixtures of known and unknown hazardous substances in complex industrial wastewater. Consequently it can address some of the shortcomings of the substance-specific approach by providing a more complete picture of the ecological hazard of complex industrial wastewater discharges.

The methodology consists of a range of effect parameters that can provide direct information on the potential toxicity hazard of the complex discharge and a battery of tests to be performed on a sample of a complex waste discharge to show up potential adverse effects. Test parameters include:

- Oxygen demand.
- · Acute toxicity.
- Chronic toxicity.
- Mutagenicity.
- Bioaccumulation potential.
- Persistence.

2.2.3 The National Toxicity Monitoring Programme (NTMP)

The National Toxicity Monitoring Programme (NTMP) is a "status and trends" monitoring program of water quality relating to toxicants and toxicity (DWAF, 2005). Through the use of various aquatic toxicity test methods it aims to report on both the potential for toxic effects to selected test organisms and on potentially toxic substances in South African inland surface water resources (DWAF, 2006). The NTMP was designed in anticipation of the DWAF's Resource Classification System (Murray et al., 2004). It will play a support role and provide supplementary information to various national monitoring programmes currently being implemented by the DWA that will focus on determining resource quality objectives for South African water resources. As such, the NTMP has an important role to play in water resource management.

CHAPTER 3: REVIEW OF QUALITY ASSURANCE & CONTROL FOR TOXICITY ASSAYS

3.1 INTRODUCTION

The South African National Water Act (Act 36 of 1998) (NWA) mandates the establishment of policies and approaches to reduce and prevent degradation and to assess the quality of water resources. To comply with these requirements, the Direct Estimation of Ecological Effect Potential (DEEEP) approach for complex industrial wastewater discharge (DWAF, 2003) and the National Toxicity Monitoring Programme for Surface Waters (NTMP) (DWAF, 2005) were introduced by the Department of Water Affairs and Forestry (DWAF) to monitor discharges into surface water and the surface water itself.

The DEEEP approach will assess the ecological hazard posed by complex industrial wastewater discharges. This is necessary because it has become increasingly apparent over the last two decades that substance-specific methods and chemical data alone have not been effective in assessing the direct environmental toxicity hazard of discharges containing mixtures of substances (DWAF, 2003). It is an effect-based approach that will make use of both standard acute and chronic tests to set limits using wastewater toxicity as the control parameter. Whole effluent toxicity (WET) tests under the Clean Water Act in the United States of America (USA) (US EPA, 1994), Direct Toxicity Assessment (DTA) from the United Kingdom Environmental Agency (National Rivers Authority, 1994) and the Netherlands' Institute for Inland Water Management and Waste Water Treatment (RIZA) (Rand et al., 1995) are examples of environmental assessment methodology approaches that have been used in a regulatory context to monitor and measure environmental (water) quality objectives.

The NTMP is essentially a "status and trends" monitoring programme of water quality relating to toxicants and toxicity (DWAF, 2005). It aims to report on both the potential for toxic effects to selected test organisms and on potentially toxic substances in South African inland surface water resources (DWAF, 2006). The NTMP was designed in anticipation of DWAF's Resource Classification System (Murray et al., 2004). The NTMP will play a support role and provide supplementary information to monitoring programmes that will focus on determining resource quality objectives for South African water resources. The NTMP therefore has an important role to play in water resource management.

There is an increasing demand, driven either by legislation and/or regulatory requirements, for quality assurance (QA) and quality control (QC) in biological tests (Hale, 1998). European Union (EU) member states as well as the United States of America (USA) and Canada are required to include QA and QC appropriate systems in the design of environmental monitoring programmes (Ecologic, 2006; US EPA, 2000; Canadian Council of Ministers of the Environment, 2006) as the data generated by monitoring programmes form the basis for regulatory decisions and measures required to achieve the objectives of the various monitoring programmes. These QA and QC systems extend far beyond simply the data generated in laboratories.

Quality assurance is a continuous improvement process and is achieved through the regular auditing of the quality system (Hale, 1998). QA is a system of management and operational activities designed to ensure adequate control of quality in the data being produced. QC involves the day-to-day routine checks and calibrations needed for normal operations which provide direct quantitative measurements of data quality. Quality control also ensures the proper functioning of the test system (Report EPS1/RM, 1996).

Reliability of data is synonymous with the quality of such data. Furthermore, data quality is directly related to the QA and QC practices applied in the test laboratory. These practices are inherent in all toxicity tests (Report EPS1/RM, 1996). All toxicity tests exhibit variability (US EPA, 1991a). According to the technical support document for toxics control (US EPA, 1991a), precision and accuracy (Slabbert et al., 1998) affects toxicity test variability and there are several factors that can influence the precision of toxicity tests. These include, but are not limited to:

- Test organism age, condition and sensitivity;
- Temperature control;
- Salinity;
- pH control;
- Handling and feeding of the test organisms; and
- Training of laboratory personnel.

While standard toxicity test methods were documented for both the NTMP and DEEEP approaches (DWAF, 2005; Slabbert, 2004), QA requirements that will affect the accuracy and precision of the data that are generated are not detailed in the DEEEP method manual.

Within the NTMP, the International Standard Organisation (ISO) 9001:2000 quality management system was recommended to be applied to ensure that the objectives of the NTMP are met (DWAF, 2005). ISO 9000 is a series of QA standards that is becoming increasingly relevant to toxicity laboratories. There are five categories of standards and the required technical standards for testing laboratories are outlined in ISO 17025 (General requirements for the competence of testing and calibration laboratories).

The principles of the 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 (Murray et al., 2004). Furthermore, accreditation of the laboratories according to ISO17025:2005 (ISO/IEC SANS 17025:2005 (E), 2005) that are performing the analyses for the NTMP is envisaged when the programme is established nationwide and when nationwide analytical capacity exists.

ISO 17025 describes the minimum requirements to be followed by a laboratory wishing to be accredited to perform certain types of assays. Each accreditation corresponds to a specific standardised test performed in the laboratory, a specific analytical technique, or assays performed on a specific matrix, or a specified type of sample (ISO/IEC 17025:2005 (E), 2005). ISO has an extensive list of standard acute toxicity tests that have been validated and have been verified with inter-laboratory comparisons.

In order for the application of the proposed toxicity tests to be successfully implemented for pollution control of water resources and the monitoring of water quality as set out in the NWA (Act 36 of 1998), the results generated from the toxicity tests must be legally defensible. There should be good confidence in the results, test methods, the laboratories and their staff. According to Soares and Callow (1993), the tacit of any legal device is that it must be applied uniformly, and must be demonstrable if required. Therefore, toxicity tests used for regulatory compliance must give the same result when applied to the same chemical in different laboratories and the same laboratories at different times of the year. The tests must show good reproducibility, precision and consistency (Slabbert et al., 1998).

The toxicity methods included in the DEEEP method are presented in terms of:

- The test environment:
- Materials, equipment and reagents;
- Test organism (breeding and maintenance);
- Test procedure:
- Data analysis and expression of results;
- · Test precision of results;
- Test report format, as well as related issues such as:-
- Sample collection, transport and storage; and
- Waste disposal.

However, quality requirements pertaining to standard operating procedures for all the above and training of new staff to comply with minimum requirements for accreditation purposes according to the International Standard ISO/IEC 17025 are not addressed in the DEEEP document. If the DEEEP method is to be given legal standing to control and monitor point source pollution in terms of licensing and setting licensing conditions, the toxicity tests on which it are based must be standardised and accepted by the regulatory as well as scientific community (Jooste and Herbst, 2004). In addition to the lack of quality requirements, a key issue in the development of standards and criteria of the toxicity tests relates to the variability of the toxicity test results generated in South Africa and how this compares to results from the USA, United Kingdom (UK) and Europe (DWAF, 2003).

At present a large number of standardised toxicity tests are currently available in the EU, the USA and Canada. In North America the standards setting organisations are the US EPA, the American Society for Testing and Materials (ASTM) and Environment Canada (EC). EU countries mainly use methods proposed by the ISO while the Organisation of Economic Cooperation and Development (OECD) proposes to implement both standard and national methods (Vosylenié, 2007). It is worthwhile noting that QA and QC requirements may vary between toxicity test methods and standards-setting organisations (**Table 3.1**) (Hoffman et al., 2002).

Quality assurance programmes for toxicity tests include QC as well as:

- Daily laboratory operation;
- Source and condition of the test organisms;
- Implementation of methods;
- Training of personnel;
- · Sample collection, handling and disposal;
- Co-ordination of testing;
- Procedures for data collection;
- Analysis; and
- Reporting (Report EPS1/RM, 1996).

In addition to the QA and QC aspects, a QA programme also should include audits and site visits that can adequately assess the QA programme. Certification and accreditation provide recognition of a laboratory's qualification and competence.

Table 3.1. Quality assurance requirements for toxicity tests from selected standards-setting organisations

QA Requirements	EPA	ASTM	EC	ISO	OECD
Sample collection and handling	✓	✓	✓	✓	
Facilities	\checkmark		\checkmark	\checkmark	
Equipment	\checkmark	✓	\checkmark	\checkmark	
Test organisms	\checkmark	✓	\checkmark	\checkmark	\checkmark
Laboratory water used for culturing and test water dilution	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
Effluent sampling and sample handling	\checkmark		\checkmark	\checkmark	
Test conditions	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
Quality of test organisms	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark
Food quality	\checkmark		\checkmark	\checkmark	
Acceptability of acute toxicity test results	\checkmark	\checkmark	\checkmark	\checkmark	✓
Analytical methods	\checkmark		\checkmark	\checkmark	
Calibration and standardisation	\checkmark		\checkmark	\checkmark	
Replication and test sensitivity	✓		\checkmark	✓	\checkmark
Documenting ongoing laboratory performance	✓		\checkmark	✓	
Variability in toxicity test results	\checkmark		✓	\checkmark	✓
Reference toxicants	\checkmark	\checkmark	✓	\checkmark	✓
Demonstration of acceptable laboratory performance	✓		✓	✓	
Measurement of estimation of uncertainty			✓	✓	\checkmark

3.2 UNITED STATES OF AMERICA

3.2.1 Introduction

The US Congress has enacted laws calling for limits on chemical exposures that "provide an ample margin of safety to protect public health" (Clean Air Act, 1963), "assure protection of public health" (Clean Water Act, 1977) provide "a reasonable certainty that no harm will result" (Food Quality Protection Act, 1996); and "adequately assure, to the extent feasible, on the basis of the best available evidence, that no employee will suffer material impairment of health or functional capacity" (Occupational Safety and Health Act, 1970). National agencies implement the statutes by promulgating standards and adopting guidance levels that limit people's exposure to chemicals. The standards and guidance levels are often developed through human health risk assessment and toxicity testing provides much of the information needed to characterise the nature and extent of the risk so that appropriate risk management actions can be taken (NRC, 2006).

3.2.2 Environmental Protection Agency (EPA)

Quality Assurance participation by EPA and EPA-supporting organisations has been mandated since 1979. The EPA order CIO 2105.0 (formerly 5360.1 A2) "Policy and program requirements for the mandatory agency-wide quality system" provides requirements for the conduct of quality management practices, including QA and quality control QC activities, for all environmental data collection and environmental technology programmes performed by or for the EPA (US EPA, 2000).

The guidelines in the "Manual for the evaluation of laboratories performing aquatic toxicity tests" (EPA, 1991b) were developed for use by the US EPA regional and state programmes as well as the National

Pollutant Discharge Elimination System (NPDES) compliance monitoring programme. The manual provides standardised procedures for conducting audits and evaluations of laboratories performing toxicity tests of effluents and surface waters. It also provides comprehensive guidelines for quality assurance and control.

In 2006, in a bid to realise the goal of a national accreditation programme, the National Environmental Laboratory Accreditation Conference (NELAC) and the Institute for National Environmental Laboratory Accreditation (INELA) formed The NELAC Institute (TNI). The purpose of TNI is to foster the generation of environmental data of known and documented quality through an open, inclusive, and transparent process that is responsive to the needs of the environmental laboratory and monitoring communities. Accreditation of environmental laboratories is done according to the ISO 17025 standard (US EPA, 2003).

In the USA, the EPA has published numerous written protocols for measuring toxicity using a variety of test organisms (US EPA, 2002a). The quality assurance procedures within EPA toxicity methods which are addressed include requirements for:

- Facilities:
- Equipment;
- Test organisms;
- Laboratory water used for culturing and test water dilution;
- · Effluent sampling and sample handling;
- Test conditions;
- Quality of test organisms;
- Food quality;
- Acceptability of acute toxicity test results;
- Analytical methods;
- Calibration and standardisation;
- Replication and test sensitivity:
- Documenting ongoing laboratory performance;
- Variability in toxicity test results;
- · Reference toxicants; and
- Demonstration of acceptable laboratory performance.

As an example, the EPA document EPA-821-R-02-012 (US EPA, 2002) describes acute toxicity tests for use in the NPDES Permits Program (mandated by the Federal Water Pollution Control Act of 1972) (Federal Water Pollution Act., 1972) to identify toxicants in effluents in receiving waters (Vosyliené, 2007). While the experimental design of many laboratory test methods are quite similar, the primary difference among test methods is that they are species specific for different geographic areas or regulatory programmes. Consequently, this leads to differences in the need for species-specific feeding, culturing, testing and end-point measurement requirements.

3.2.3 American Society for Testing Materials (ASTM)

The EPA was instrumental in the formation of the ASTM Committee that is responsible for the development of more than 100 ASTM standards dealing with methods for assessing the fate and effects of contaminants released into the environment. In 1980, the "Guide for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians" was published. This standard was a landmark standard and is often described as the fundamental document for aquatic toxicity testing worldwide. The original guide has subsequently been updated (ASTM, 1996). Quality assurance procedures that are addressed within ASTM toxicity methods are shown in **Table 3.1**.

3.3 CANADA

The Canadian Environmental Protection Act was promulgated in March 2000 and its objective is to prevent pollution and protect the environment and human health. The act requires that toxicity testing for registration of new substances conform to OECD standards of Good Laboratory Practice (GLP) (Report EPS1/RM, 1996).

In order for the toxicity tests to meet regulatory requirements, the Canadian Inter-Governmental Aquatic Toxicity Group proposed the development and standardisation of a set of single species aquatic toxicity tests. The Environmental Protection Series describes the recommended methods for aquatic organisms and deals with the use of reference toxicants as well as reference methods for toxicity test precision control (Report EPS 1/RM/24, 1992). The general QA requirements for toxicity tests used in Canada are summarised in **Table 3.1**.

The Canada-wide Framework for Water Quality Monitoring aims to enhance water resource management by serving as a guide to jurisdictions in the development and implementation of water quality monitoring programmes in Canada (Canadian Council of Ministers of the Environment, 2006). For the protection of aquatic life, acute (as well as chronic) toxicity to aquatic life is suggested as a variable of concern. It is recommended that, where possible, standard toxicity test methods are employed and that currently acceptable laboratory practices of exposure and control must be utilised (Canadian Environmental Quality Guidelines, 1999). The framework calls for the analytical laboratories to maintain full documentation on QA/QC procedures, be prepared to undergo proficiency testing for the variables in question and make any test results available. Only analytical laboratories accredited for specific analytical tests under the Standards Council of Canada, the Canadian Association of Environmental Analytical Laboratories (CAEAL) or an equivalent accreditation body may be used.

The CAEAL has a programme for accrediting toxicological laboratories (Report EPS1/RM, 1996). It requires a site visit, participation in performance testing, and an audit every two years. The CAEAL accredits analytical laboratories in accordance with the ISO 17025 Standard.

3.4 EUROPEAN UNION (EU)

The European Union's Water Framework Directive (WFD) aims to protect and enhance the quality of all inland and coastal waters within defined river basin districts in Europe by improving and integrating the way water bodies throughout Europe are managed. All EU member states are required to design monitoring programmes to comply with the requirements of the Water Framework Directive (WFD) (Ecologic, 2006). In order to achieve the environmental objectives of the WFD, EU monitoring laboratories need to have appropriate QA programmes in place to ensure the reliability of the data being generated.

Standards for water quality and dangerous substances are issued by the EC Directives (Slabbert et al., 1998). Toxicity testing of environmental agents to provide data for human health risk assessments occurs in the EU under Commission Directives 93/67/EEC and 98/8/EC and Commission Regulation 1488/ 94, which directs the risk assessment of new substances, existing substances, and biocidal products (NRC, 2006).

Directive 93/67/EEC lays down the principles for assessment of risks to man and the environment of substances notified in accordance with Council Directive 67/548/EEC. Directive 98/8/EC provides a framework of rules that apply to the marketing of biocidal substances and products and stipulates a high level of protection for humans, animals and the environment (NRC, 2006).

The 7th Amendment to Directive 67/548/EEC (1992) which deals with risk assessment of new chemical substances was adopted by the European Commission in 1993 and requires the following set of toxicity tests to be conducted:

- Acute toxicity for fish;
- · Acute toxicity for daphnia;
- Algal growth inhibition; and
- Bacterial inhibition (Furlong, 1995).

The methods, where available, are based on those recognised and recommended by, in particular, the OECD. When such methods were not available, national standards or scientific consensus methods must be adopted.

REACH is a new European Community Regulation on chemicals and their safe use (EC 1907/2006, 2006). It deals with the Registration, Evaluation, Authorisation and Restriction of Chemical substances and came into force on 1 June 2007. The main aims of REACH are to improve the protection of human health and the environment from the risks that can be posed by chemicals, the promotion of alternative test methods, the free circulation of substances on the internal market and enhancing competitiveness and innovation. Note that the adoption of alternative methods for assessing the hazard associated with the usage of chemicals is strongly recommended especially where it will lead to a reduction of animal testing.

3.5 ORGANISATION FOR ECONOMIC COOPERATION AND DEVELOPMENT (OECD)

While the OECD has published a document promoting the use of biological tests for water pollution assessment and control (OECD, 1987), specific test methods are not specified. Instead it is up to the individual countries to determine this based on their unique requirements and circumstances. Standard OECD methods address QA procedures linked to:

- Test organisms;
- Laboratory water used for culturing and test water dilution;
- Test conditions;
- Quality of test organisms;
- Acceptability of acute toxicity test results;
- Replication and test sensitivity;
- · Variability in toxicity test results; and
- Reference toxicants as summarised in **Table 3.1**.

Compliance with Good Laboratory Practice (GLP) is a regulatory requirement for the acceptance of certain results (OECD, 1999). In 1989, OECD member countries agreed that where "testing of chemicals for purposes of assessment related to the protection of health and the environment is being carried out pursuant to principles of good laboratory practice that are consistent with the OECD principles of GLP, they shall, amongst others, establish national procedures for monitoring compliance with GLP Principles, based on laboratory inspections and study audits" (OECD, 1994).

In 1990, a working group reached consensus on the role of QA as an important component of GLP. The OECD developed the GLP programme mainly for testing new products for registration. GLP guidelines are acceptable to all OECD member nations and describe the requirements for conducting and reporting a study. The level of QA in GLP studies usually meet requirements for ensuring reliable data (Report EPS1/RM,

1996). In the revised OECD Principles of GLP, a QA programme is defined as a system that is designed to assure test facility management of compliance with the principles of GLP (OECD, 1999).

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CHAPTER 4: AQUATIC TOXICITY TESTING IN SOUTH AFRICA

4.1 INTRODUCTION

In a study undertaken by Golder Associates in 2006 (DWAF, 2006) it was found that only six of the approximately twenty aquatic toxicity testing facilities were accredited. A recent study commissioned by the WRC found that there are a total of sixteen laboratories nationwide that are able to conduct toxicity testing. At present there are only four accredited aquatic toxicity testing laboratories in South Africa of which three are in situated in Gauteng and one in KwaZulu-Natal as shown in **Table 4.1** (Balfour *et al*, 2009). Furthermore the study also found that:

- The only other laboratory that can undertake ecotoxicity analyses is in Cape Town, although the laboratory is not accredited.
- It is only in Gauteng in which capacity exists to undertake toxicity tests on a routine basis.
- Accreditation is not commercially viable for the smaller facilities that undertake toxicity tests.

Table 4.1. ISO 17025 Accredited Toxicity Testing Laboratories

Laboratory	Locality	Lab. Number
Golder Associates Research Laboratory – Aquatic Toxicity Division	Johannesburg	T0384
Rand Water Analytical Services	Vereeniging	T0046
Resource Quality Services (DWA)	Pretoria	T0073
Umgeni Water – Amanzi	Pietermaritzburg	T0036

The closure of the Council for Scientific and Industrial Research's (CSIR) Pretoria aquatic toxicity testing laboratory in 2008, although not accredited will leave a significant research and development gap in the field of toxicity testing.

A workshop between toxicity testing laboratories was facilitated to discuss important issues pertaining to laboratories supplying this service. All present were requested to complete a Questionnaire (Appendix A) to determine the present status of facilities and perceptions regarding the future application of toxicity testing. The questionnaire was also sent to all known individuals and institutions involved in toxicity testing who could not attend the workshop. A list with the details of those who responded is in Appendix B. The locality of the institutions and individuals involved with toxicity testing is shown in **Figure 4.1**. It confirms the fact that the vast majority are situated in Gauteng.

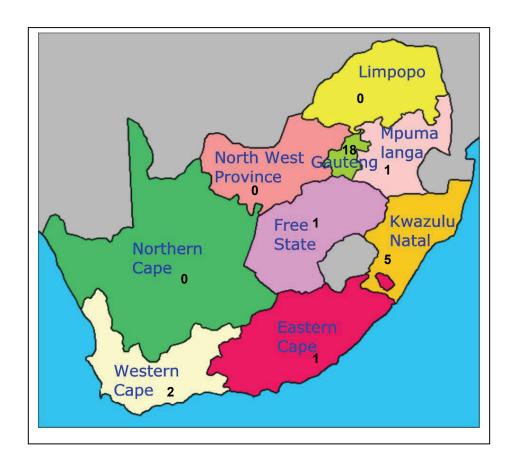


Figure 4.1. Locality of aquatic toxicity testing facilities and individuals involved with aquatic toxicity testing in South Africa, June 2009

4.2 ISSUES RAISED AT THE TOXICITY TESTING WORKSHOP

4.2.1 Accreditation and proficiency testing

There are currently only four South African National Accreditation System (SANAS) accredited facilities. From comments in the questionnaire and discussions with respondents, in particular the smaller service providers, it was made abundantly clear that cost implications and the uncertain application of toxicity testing in the near future are the main reasons for the reluctance to invest in capacity building and accreditation. It seems that it might not be financially viable for small laboratories to participate in accreditation. Good Laboratory Practice (GLP) compliance may be more practical and financially reasonable. Respondents who indicated current involvement with proficiency testing schemes (PTS) also indicated the need for additional proficiency testing schemes with *Danio rerio* and *Selanastrum capricornutum*. The majority of those already involved with proficiency testing schemes cited participation in the Rand Water *Daphnia pulex* proficiency testing scheme. Some of the respondents who were not currently participating in proficiency testing schemes based their reasons on issues such as uncertainty regarding the schemes which are available and cost implications. In addition the advantages of participating in such schemes were not known.

4.2.2 Volume of samples and financial viability

Responses in this regard indicate relatively low volumes of toxicity testing at present. Financial viability at levels of between 20 and 50 samples per month would certainly facilitate capacity building if the demand for the service would increase and remain viable.

4.2.3 Major clients and consultant services

Major water users and dischargers of effluent are already requiring the services and advice of experts in the field of toxicity testing. Advice is mainly provided by smaller enterprises which provide consultancy services. What should be noted, however, is the role played by academic institutions. Apart from their primary educational and training objectives these institutions can undertake toxicity testing in areas where there are no service providers and they can facilitate the establishment of private enterprises.

4.2.4 Staff (human resources)

Looking at the number of analysts and other personnel involved in toxicity testing it would seem that trained and experienced personnel could be a limiting factor. Capacity building and exploring the work market would require time and it is therefore imperative that the timetable and scope of legislative and policy interventions be made available.

4.2.5 Assistance and interaction

The responses as regard to interaction between stakeholders, participation in policy formulation and research and networking were extremely positive. Most of the respondents are subscribed to the "Aquatox Forum" which actively promotes the interests and views of those involved in this field. Respondents indicated that they would be amenable to assistance from experts in the field of aquatic toxicity and accreditation. Facilitation of this would largely occur on an *ad hoc* basis although respondents have indicated that goal-oriented workshops and training sessions would be most valuable for them to attend.

4.2.6 Research needs

The following research needs were identified:

- Effect of anti-retrovirals on wastewater treatment works effluent quality.
- Biomarkers in aquatic toxicity tests.
- Chronic/sub-chronic test method development.
- Effluent sample inclusion in a PTS.
- New methods for industry that are quick and reliable.
- Practical applications of toxicity tests for industry.
- Sediment toxicity method development.

4.2.7 DEEEP involvement and application in the field

The majority of the participants are aware of DEEEP and indeed are in possession of the policy document. With minor exceptions most are capable of, and presently undertaking DEEEP specified toxicity testing.

4.2.8 Perceptions and views

Responses in this regard clearly indicate that although the majority of participants regard toxicity testing as an excellent instrument to be used in water resource management, they are disappointed with its current application in South Africa.

Respondents identified the following issues that must be addressed to ensure the successful implementation of toxicity testing in accordance with the DEEEP objectives:

- Accreditation of participating institutions. This is a costly and cumbersome process and the investment is not seen as worthwhile under present circumstances.
- The absence of standardised methods and toxicity test standards are detrimental to service quality and interpretation of results on which clients rely upon.
- There is some doubt whether adequate expert human resources are available at this stage.
- Funding and training assistance to establish operational units would enhance service delivery on a national scale.
- Some respondents indicated that client understanding of toxicity testing and application thereof is not up to standard.
- No testing facilities in the immediate vicinity of the respondents and problems with couriers if samples are to be transported over a long distance.

4.3 DISCUSSION

In the present legislative vacuum there is no incentive for service providers to invest in capacity building. It is vital that time scales for the full, or phased-in implementation of DEEEP be determined as a matter of urgency. An authoritative decision to implement would provide the assurance that toxicity testing would be financially viable and it is anticipated that the necessary capacity will be made available. It is anticipated that the development of standardised methods, test standards and administration of the venture would take at least one year to complete and this will provide time to initiate capacity building programmes. Apart from its core responsibility to develop policy guidelines and prescriptions, the Department should also consider initiating job creation and developmental ventures such as indicated in Presidential and other programmes. In this regard the academic institutions could play a major role.

There is a need for all toxicity testing laboratories, whether they are government or private laboratories, to support each other and address issues hampering routine aquatic toxicity testing. This can be done by establishing networks between toxicity testing laboratories as demonstrated by the role that the Aquatox Forum fulfilled in the past.

For the past 10 years the Aquatox Forum, an interest group for people involved with or with an interest in aquatic toxicity testing has addressed many of the needs expressed by the respondents for the successful implementation of toxicity testing. Working Groups were formed addressing the following topics:

- Standardisation of aquatic toxicity test methods.
- Emergency research and development in biotoxicology.
- Implementation of toxicity tests.

The Forum also arranged seminars and workshops addressing, amongst others, the following topics:

- Management implementation.
- Environmental evaluation.
- Relevant research and development.

- Biological effects assessment to manage complex wastewater discharges.
- Toxicity test methodology.

On two occasions a Canadian expert on toxicity testing, Dr Christian Blaise, was the guest speaker. Members of the Forum were also involved with the hosting of the 9th International Symposium of Toxicity Assessment in Pretoria in 1999. A member of the Forum also initiated and ran a proficiency testing scheme for the Daphnia acute toxicity test. These activities were funded by attendance and membership fees, exhibition fees and sponsorships by members. Because most members are located in Gauteng all the activities were held in Gauteng. This prevented some members attending because of the associated costs. All the activities are run by members holding full-time jobs which reduce the amount of time and effort available for the promotion of the activities of the Forum. Although DWA was represented on the committee in the past, more involvement is necessary from the Department at national and regional level, especially those who will be responsible for the implementation of aquatic toxicity testing.

CHAPTER 5: DRAFT IMPLEMENTATION PLAN

5.1 METHODOLOGY

To obtain the views of government officials responsible for fulfilling the requirements of the NWA in terms of water resource management, DWA officials (Appendix D) involved with water quality management were requested to complete a questionnaire (Appendix C) to determine the success of the implementation of the DEEEP approach thus far. Academics (Appendix E) doing research in the fields of water quality management and monitoring were requested to complete the same questionnaire. Information gained from the survey and the toxicity testing laboratory surveys were used to identify key success factors for the implementation of routine aquatic toxicity testing.

5.2 KEY SUCCESS FACTORS

5.2.1 Development of toxicity test standards

One of the fundamental requirements of regulatory compliance monitoring is that standardised test methods be used for assessing compliance with regulatory limits. Toxicity tests used for regulatory compliance must provide the same result when applied to the same chemical in different laboratories and the same laboratories at different times of the year. By making use of a standardised test method, not only is the precision of the data generated increased but the comparison of data and results are facilitated. In essence the scientific integrity of the results and data provides a credible basis to withstand the rigours of a legal inquiry. This implies that together with Toxicity Test Standards, Limits for Toxicity Tests should be established. The DEEEP document suggests that limits should be evaluated. In South Africa, no limits currently exist and as such realistic limits must be set. This necessitates the development of water guidelines where toxicity levels should also be addressed.

Revised water guidelines should be put in place and must be situation specific. Risk determination must also be taken into account.

According to Soares and Callow (1993), the tacit assumption of any legal device is that it must be applied uniformly and must be demonstrable if required. The tests must show good repeatability, reproducibility, precision and consistency (Slabbert et al., 1998).

Laboratory accreditation and associated activities provide some assurance regarding the technical proficiency and competence of a laboratory to perform toxicity tests to an international standard. The establishment of a National Toxicity Testing Laboratory Accreditation Programme (NTTLAP) is needed to ensure that national accreditation of laboratories to the ISO/IEC 17025 standard is achieved. The South African National Accreditation System (SANAS), the only accreditation body in South Africa, is required to oversee such a programme. The NTTLAP will include the compulsory participation of accredited laboratories in a PTS upon which accreditation is contingent on the satisfactory performance of the laboratory as well as regular assessments by the accreditation body, SANAS.

5.2.2 Adequate regulation

The Canadian Government requires that specific reference methods be used when regulatory testing is required while Australia, New Zealand, the USA and EU countries have specific frameworks and regulations (NWQMS, the CWA, the WFD, etc.) that mandate the use of toxicity tests. Unless specific statutory requirements for regulatory testing are established, enforcement of such requirements becomes impossible and the protection of natural resources and sustainable use thereof cannot be realised.

Policy and regulations must be integrated

5.2.3 Licence conditions

It is essential that licensing conditions regarding ecotoxicity be imposed and that ecotoxicity testing be included in the licences. Without a driver for toxicity testing in a regulatory context, implementation of routine toxicity testing becomes useless. General licensing conditions must include points with reference to:

- Sampling, monitoring, analysis and/or investigation of specific areas of concern regarding aquatic toxicity (typically final effluents released into surface waters).
- That the Licencee (he/she) shall, unless otherwise specified in this Licence:
 - Carry out all preservations and analyses of liquid samples in accordance with the methods prescribed in the standard methods as prescribed by the regulatory authority.
 - Ensure that all analytical determinations are undertaken by an accredited laboratory, and
 - Ensure that the discharger carries the cost of analyses.

Type of effluent as well as grade of receiving water must be taken into consideration when determining licence conditions.

Industries must be made aware of the need for toxicity testing and the facilities where such testing can take place must also be made known. Potentially a problem with ownership of data exists. However, if toxicity is included in a discharge/water use license, then the data become the property of DWA and therefore automatically enter the public domain. The mere act of paying for such analyses and subsequent data does not imply direct ownership. If copyright of reports vests in the testing laboratory or a consultant, then the data belong to that testing laboratory or consultant, unless otherwise dictated in a formally binding contract. In these instances, access to such information must be obtained through a Promotion of Access to Information Act (PAIA) application. Refer to Appendix F for an example of text to be included in the licence condition.

5.2.4 Compliance criteria

In order to determine whether or not a licencee may/may not be compliant with the relevant regulations, it is necessary to measure the results of the toxicity tests against the guidelines and toxicity limits decided upon by the regulatory authority. Specific guidelines for the expression of data and handling of results must be readily available. It is up to the regulatory authority to determine whether NOECs, LC/EC_{50s} should be used. TUs or narrative descriptions, etc. are sufficient in order to fulfil the requirements of data reporting to determine compliance.

The inclusion of at least one toxicity parameter with a set toxicity standard will aid compliance and regulation.

5.2.5 Legal action

The course of action to address non-compliance must be determined. It is necessary to engage with interested and affected stakeholders to determine what the expectations of the stakeholders are in terms of legal action for non-compliance. Penalties by means of fines may be imposed or revoking of licences may be considered in the case of non-compliance.

Enforcement officers are vital to assess compliance. Officers must be hired, trained and actively involved in compliance enforcement.

5.2.6 Strengthening national capacities

Capacity building refers to the process by which individuals, groups, organisations, institutions and countries develop their abilities, individually and collectively, to perform functions, solve problems and achieve objectives (Baillie et al., 2008).

The objective of the Implementation Plan is to realise nationwide routine toxicity testing by DWA. In order to achieve that, capacity to perform the toxicity tests needs to be established. This includes a competent human resource component as well as organisations (laboratories) that are accredited to do such toxicity testing.

According to Balfour et al. (2009), accreditation of toxicity testing laboratories must be driven by increased emphasis placed by DWA on the importance of credible water quality test results and increased municipal budgets for monitoring water quality.

Further recommendations from Balfour et al. (2009) include:

- Human resource challenges should be addressed via in-service training for graduates.
- Regional training courses will make training more affordable and accessible.
- DWA personnel must be capacitated to understand and interpret results submitted to them.
- Strategic partnerships with SANAS and the National Laboratory Association (NLA) and water boards must be established.
- DWA must implement their proposed laboratory strategy that will require laboratories undertaking
 water quality testing for municipalities to be "Approved" per method if the results are to be accepted
 by the DWA.
- A communication strategy that includes the general public, laboratories, municipalities and relevant government departments will aide in raising the profile of water quality testing.

5.3 PHASED APPROACH TO NATIONAL IMPLEMENTATION

5.3.1 Process overview

Firstly, it may be necessary for the national office of DWA to appoint a National Coordinating Manager to ensure successful implementation occurs and to serve as a central point of communication for industries and DWA regions as well as representatives from laboratories. Implementation of routine toxicity testing by DWA

should begin with an enabling phase to develop stakeholder participation. Stakeholders typically would include the DWA regions, regional laboratories and industries. Raising awareness and obtaining buy-in from stakeholders can be achieved by means of workshops and road shows. The various Catchment Management Agencies (CMAs) operating in various regions can be used as a starting point to raise awareness both at stakeholder and industries level. Furthermore, it is strongly recommended that a phased approach, similar to that adopted by the NTMP and River Health Programme (RHP) be adopted.

A "National Implementation Process" as in the context of the NTMP, is defined as that series of actions required to set up and sustain a successful national monitoring program. **Figure 5.1** shows the proposed steps in the process (adapted from Murray et al., 2004).

5.3.2 Select DWA region

In order to ensure ongoing routine toxicity testing in South Africa, it is recommended that implementation in the various DWA regions of South Africa be phased in. The regional water quality managers should be engaged unless other relevant persons are indicated. In addition, all existing licence/permit holders should be included in implementation. The regions, as per DWA divisions, can be maintained as they are rather than phasing-in per provincial area. This helps facilitate regional buy-in. **Table 5.1** shows the present DWA divisions for the regions including the corresponding Water Management Area (WMA).

It is recommended that implementation be initiated in Gauteng. The capacity for analyses exists in terms of private laboratories that are accredited for toxicity testing as well as the number of non-accredited toxicity testing laboratories. Industries should be sensitised to the need that toxicity testing is required and that toxicity will be included into licences upon licence application and review. The use of road shows is a good way of bringing the message across. Gazetting the requirement for toxicity testing to be included into licences raises the awareness of industries to toxicity testing.

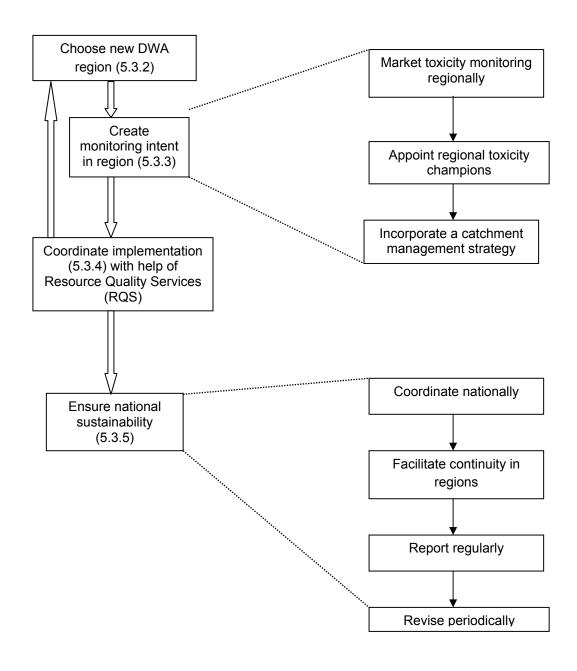


Figure 5.1. Phased National Implementation Process for routine aquatic toxicity testing (adapted from Murray et al., 2004)

Table 5.1. DWA regional divisions, water management areas (WMAs) and method accreditation status of regional laboratories

Regional Divisions	Water Management Area	Accredited Regional Laboratory
Eastern Cape	Mzimvubi to Keiskamma	No
	Fish to Tsitsikamma	
Free State	Middle Vaal	No
	Upper Orange	
Gauteng South	Upper Vaal	Yes
KwaZulu-Natal	Usuto to Mhlatuze Thukela	Yes
	Mvoti to Umzimkulu	
Limpopo	Limpopo	No
	Luvuvhu & Letaba	
Mpumalanga	Olifants	No
	Nkomati	
Northern Cape	Lower Vaal	No
	Lower Orange	
North West & Gauteng North	Crocodile West and Marico	No
Western Cape	Gouritz	No
	Olifantsdoorn	
	Breede	
	Berg	

5.3.3 Creating monitoring intent in regions

The National Coordinating Manager through the further appointment of regional champions must ensure that the regions and the relevant Catchment Management Agencies (CMAs) in the accompanying WMAs have the capacity to implement toxicity testing routinely. This will include providing a basic introduction to toxicity and toxicity monitoring. Analytical facilities in the regions should be encouraged through their interactions with the DWA (national/regional) to become accredited for toxicity testing and the "Guideline for the Accreditation of Routine Aquatic Toxicity Testing Laboratories" prepared in conjunction with this document must be made available to the regions and laboratories.

The regional champions will require the support of the CMAs to ensure successful implementation of routine aquatic toxicity testing in a specific region. As was recommended in the NTMP, it is important that toxicity monitoring and the requirements for toxicity testing reach the WMA through the catchment management strategy of the CMA. During the implementation phase the discharger/polluter's interests should also be taken into consideration. Successful water resource management is dependent on cooperation among all spheres of government and the active involvement of water users and other organisations and interested and affected stakeholders (NWA, 1998). In order to ensure buy-in from the effluent dischargers, leadership in the

implementation must come through Business Chambers, Industrial Forums, Local Government Forums (e.g. South African Local Government Association (SALGA)), etc.

5.3.4 Coordinated implementation

To ensure the successful implementation of routine toxicity testing by the DWA in all regions of South Africa, it is vital that the feedback gained from the Regional Champions via the CMAs to the National Coordinating Manager be fully assessed

5.3.5 Ensuring national sustainability

In addition to addressing feedback from the CMAs, the envisaged National Coordinating Manager must also be able to engage with the policy makers and legislative arm of the DWA. Without adequate legislation and enforcement, policy is meaningless. Likewise, regional capacity must be at a level where regional enforcement is possible. Additionally, succession planning in the regions as well as at a national level is required in order to ensure ongoing implementation in light of staff turnover. As with any monitoring programme, review of data information gained during the monitoring process, as well as programme design elements, is required from time to time.

5.4 INFORMATION EXCHANGE

Under the Promotion of Access to Information Act (Act no 2, 2000) information contained in any national monitoring system established in terms of the Water Act, must be made available upon written request. While the national implementation of routine toxicity testing of effluents is not strictly speaking a monitoring programme like the NTMP, the National Microbial Monitoring Programme and the National Chemical Monitoring Programme etc., the toxicity information generated will become the property of the DWA even though the licencee has paid for the toxicity tests. The information can be stored on the Water Management System (WMS) and in order to manage the nation's resources, reports dealing with toxicity data will also be provided. An environmental registry of toxicity data may be considered.

5.5 TECHNICAL ASSISTANCE

The need for technical assistance is foreseen with regards to the setting up of analytical facilities and such facilities seeking accreditation in terms of regulatory requirements. The "Guideline for the Accreditation of Routine Aquatic Toxicity Testing Laboratories" is aimed at facilitating accreditation of laboratories for toxicity testing. Furthermore, presentations on introductory toxicity basics will add value and go towards creating capacity in regions to implement routine toxicity testing. Networking between laboratories that have already achieved accreditation for the purpose of toxicity testing and those laboratories still seeking accreditation status should be established and this will foster a culture of cooperation between laboratories in order to achieve national objectives. The establishment of a core analytical laboratory is recommended to act as an information hub for laboratories that will participate in toxicity testing for regulatory purposes. The Resource Quality Services Laboratory or another such laboratory/institution (e.g. South African National Accreditation System, SANAS) could provide technical training in collaboration with the NLA. This training must be made available to the DWA regions.

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CHAPTER 6: SUSTAINABLE NETWORKS BETWEEN LABORATORIES

6.1 INTRODUCTION

The aim of creating a network between toxicity testing laboratories in South Africa with administrative and financial support from the Department, should be to sustain and improve the quality of aquatic toxicity testing in South Africa and assist with the implementation of routine toxicity testing as required by the DEEEP and the NTMP. This could be achieved through:

- Ensuring the comparability and validity of aquatic toxicity analyses performed by laboratories in South Africa.
- Encouraging a commitment to data integrity, accessibility, and interoperability.
- Facilitating a national information exchange on methods and other technical references.

Before this can be achieved, the toxicity testing community in South Africa must have a working reference framework that includes:

- Validated standardised toxicity tests.
- A quality manual for toxicity testing laboratories describing procedures and protocols.
- Laboratory performance evaluation studies in the form of PTSs that are administrated by an accredited laboratory. Technical advice, reference samples and training also need to be provided.
- Laboratory assessments that take the form of Quality Audits (ISO 17025) to assess the testing facilities' compliance to their proposed quality management and competence of the staff to execute the analytical methods.

The framework that has been proposed is similar to the framework adopted by the United Nations Global Environment Monitoring System (GEMS) / Water Programme (Environment Canada, 2009).

Once the framework is in place a Regional Laboratory Network (RLN) must be developed. This RLN will include the National Resource Quality Services (RQS) Laboratory. The advantages of creating such a laboratory network are:

- Improvement of Quality Assurance through accreditation and proficiency testing schemes, implementation of validated, standard operating procedures.
- Training for laboratory personnel.
- Promotion of national self-sufficiency and sustainability of laboratory services.
- Cost benefits in respect of competitive toxicity testing costs.

6.2 PROPOSED APPROACH

The following is adapted from Loko (2008).

6.2.1 Regional Laboratory Network (RLN)

The establishment of regional laboratory networks will address the problems associated with lack of capacity and organisational infrastructure by feeding back into the national objective of the nationwide implementation of routine aquatic toxicity testing.

A RLN will assist with developing regional capacity by facilitating regional cooperation and collaboration with regional government. It also aides in developing regional laboratory expertise that can again be fed back into the national pool of expertise via the RQS laboratory. Furthermore, the coordination and harmonisation of the regional government objective can be realised. Training and exchange of personnel with other reference or associated laboratories in the regional and subsequently, the national laboratory infrastructure, can occur. This further promotes the exchange of scientific data and technical knowledge.

6.2.2 Functions of a Regional Laboratory Network

The RLN shall have a coordinating and technical support role that will:

- Provide consistency and rigour in methodology.
- Enable local regional testing facilities to share technical expertise and information.
- Allow transparency and confidence in results generated by the testing laboratories.
- Improve the quality of aquatic toxicity testing and interpretation of results through standard operating procedures and by providing training and technical support to technical personnel.
- Encourage complimentary testing of environmental samples (catchment specific) in the region for inclusion into a regional database thereby promoting the use of ecotoxicity data in catchment management on a regional scale. This should also aid decision making on a regional, provincial and national level.

6.2.3 Factors contributing to long term sustainability

In order for the RLNs to be sustainable, effective funding models should be developed and implemented to support the initiatives of regional laboratories. This can be realised through the income stream generated from analyses as well as being funded by regional government. Various testing laboratories that took part in the 2009 *Toxicity Testing Status Quo* Survey indicated that unless there is a firm commitment from government to implement toxicity testing in South Africa through various legislative needs, a toxicity testing facility will not be financially viable. At present, samples for testing mainly come from the public, private organisations and research-funded projects. Financial viability at levels of between 20 and 50 samples per month on the other hand would certainly facilitate capacity building and also sustainability of the laboratories if the demand for such a service increases and remains viable.

Adequately trained and experienced personnel could be a limiting factor for sustainable laboratory networks in South Africa. Capacity building and exploring the work market would require time and it is therefore imperative that the timetable and scope of legislative and policy interventions be made available. As a commitment to ensuring that toxicity testing in South Africa is implemented and sustainable, regional government (DWA) could provide funding to the RLN via a central administrative function of the RLN.

6.2.4 Links to a National Regional Laboratory Network

The proposed links of the regional laboratory network with National Government (DWA), SANAS, NLA and Regional Laboratories are shown in **Figure 6.1**.

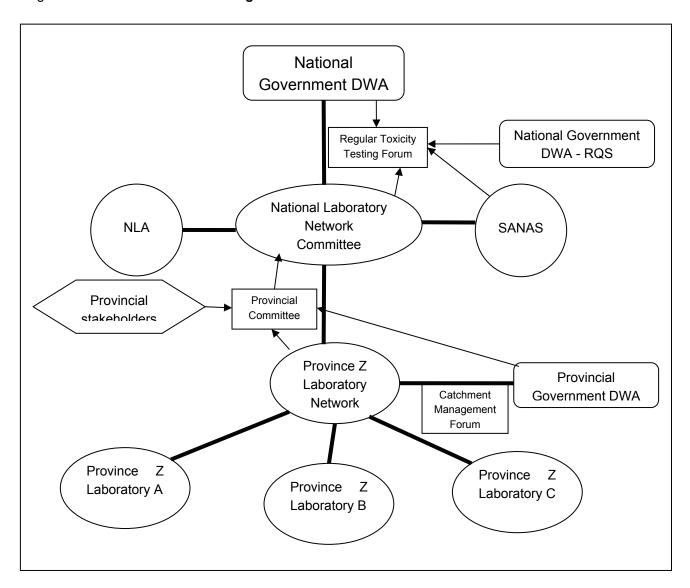


Figure 6.1. Links and communication structure of the national regional laboratory network for a province Z

It is proposed that each province of South Africa is regarded as a Region. Every Region (total of 9) will act to coordinate the toxicity testing facilities in its Region. The Regions will report to their respective regional government structures which could be facilitated through the Catchment Management Forums. Through collaboration with the South African National Accreditation System (SANAS) and the National Laboratory Association (NLA), via the National Laboratory Network Committee, the Quality Management Function of the Regional (provincial) Laboratory Network (RLN) will be achieved. The RLN reports to the national DWA via the National Laboratory Network (NLA) and communicates the national objective to each of its regional members.

6.2.5 Communication structure of the National Regional Laboratory Network (NRLN)

The various Regional Laboratory Networks report to the NRLN Committee via Regional Committees (RCs). The RCs are comprised of representatives from the laboratories in their respective regions. This could be further representative of the various local municipalities in the provinces that are represented by the regions. Australia's Animal Health Laboratory Network (AHLN) (Australian Government, 2007) has similar regional committees that are also made up of representatives for every university, private commercial sector and non-governmental organisations (NGOs). A representative of the regional government DWA must also be present on the RC.

The NRLN is made up of a representative of every one of the RCs together with a technical advisor from SANAS and this group communicates with the national DWA office via a nominated member who acts on behalf of the NRLN. The nominated member liaises with national government at a forum for national coordination in terms of regulatory toxicity testing and requirements from the regional laboratories that have been identified. A nominated representative from the national DWA toxicity testing laboratory must be present at the forum. Issues that could be included for discussion at such a forum would include:

- Quality assurance.
- New test development.
- Validation of standard operating procedures.
- Development and maintenance of a national proficiency testing scheme.
- Organisation and promotion of training opportunities.
- Technical advice relating to aquatic toxicity testing.

CHAPTER 7: CONCLUSIONS

- The successful implementation of routine toxicity testing nationwide will depend on the interactive collaboration between DWA, toxicity test service providers, industry and the general public. Structures to encourage this collaboration will thus have to be put in place.
- Although the participants in the laboratory questionnaire were positive about the planned implementation of routine toxicity testing, the present legislative vacuum provides no incentive for service providers to invest in capacity building (human resources and accreditation of test methods). An authoritative decision to implement would provide the assurance that toxicity testing would be financially viable (20 to 50 samples per month) and it is anticipated that the necessary capacity will be made available once the time scales for the full or phased-in implementation of the DEEEP is available.
- DWA is responsible for including ecotoxicity guidelines and ecotoxicity testing in licensing conditions
 for effluent discharge. To enforce compliance with these licensing conditions, DWA personnel must
 be capacitated to understand and interpret results submitted to them. Succession planning in the
 regions as well as at a national level is required in order to ensure ongoing implementation in light of
 staff turnover.
- Two-way communication between people in the regions monitoring compliance with licensing conditions, i.e. enforcement officers and catchment agencies monitoring water quality, and the National DWA Coordinating Manager will ensure constant assessing of the success of the implementation strategy and allow timeous interventions.
- A communication strategy that includes the general public, laboratories, municipalities and relevant government departments will aid in raising the profile of water quality testing.
- In case of the full national implementation of routine toxicity testing, a shortage of capacity (i.e. competent human resource component and accredited laboratories) to supply these services will exist. To sustain and improve the quality of aquatic toxicity testing in South Africa and assist with the implementation of routine toxicity testing as required by the DEEEP and the NTMP, DWA will have to implement their proposed laboratory strategy requiring laboratories undertaking water quality testing for municipalities to be "Approved" per method if the results are to be accepted by the DWA.
- Quality guidelines for toxicity testing laboratories covering test methods, analyses of test results, infrastructure and training of personnel are thus required.
- To ensure reliable and uniform test results, a quality reference framework needs to be established that includes.
 - > Validated, standardised toxicity tests.
 - > A quality manual for toxicity testing laboratories describing procedures and protocols.
 - Laboratory performance evaluation studies in the form of PTSs that are administrated by an accredited laboratory. Technical advice, reference samples and training also need to be provided.
 - ➤ Laboratory assessments that take the form of Quality Audits (ISO 17025) to assess the testing facilities' compliance to their proposed quality management and competence of the staff to execute the analytical methods, will ensure reliable test results.

- The need for affordable human capacity building can be addressed by regional training courses and in-service training for graduates. The methods document "Guideline for the Accreditation of Routine Aquatic Toxicity Testing Laboratories" prepared during this study will assist laboratories in applying for accreditation. The establishment of a core analytical laboratory, e.g. DWA's Resource Quality Services Laboratory, can act as an information hub for laboratories that will participate in toxicity testing for regulatory purposes. This laboratory or another such laboratory or institution (e.g. South African National Accreditation System, SANAS) could provide technical training in collaboration with the NLA.
- Networking between laboratories that have already achieved accreditation for the purpose of toxicity testing and those laboratories still seeking accreditation status will foster a culture of cooperation between laboratories in order to achieve a national objective. A National Regional Laboratory Network (RLN) including the National Resource Quality Services (RQS) Laboratory will include the following advantages.
 - ➤ Improvement of quality assurance through accreditation and proficiency testing schemes, implementation of validated, standard operating procedures.
 - > Training for laboratory personnel at regional level.
 - Promotion of national self-sufficiency and sustainability of laboratory services.
 - Cost benefits in respect of competitive toxicity testing costs.
 - Allow transparency and confidence in results generated by the testing laboratories.
 - Encourage complimentary testing of environmental samples (catchment specific) in the region for inclusion into a regional database thereby promoting the use of ecotoxicity data in catchment management on a regional scale. This should also aid decision making on a regional, provincial and national level.

CHAPTER 8: RECOMMENDATIONS

- As the main focus of this project was to realise nationwide routine toxicity testing by DWA, it is vital that the contents of the two reports be conveyed to the Ministry of Water Affairs.
- If the draft implementation plan for routine toxicity testing by DWA proposed in this study is implemented, a huge demand for toxicity testing laboratories will be created in all regions of the country. New laboratories entering the market now have the "Guideline for the Accreditation of Routine Aquatic Toxicity Testing Laboratories" prepared during this study to assist in preparing the laboratories for application for accreditation but they will also have a demand for trained staff. It is thus important that the practical training of staff employed by toxicity testing laboratories be addressed in a future WRC project.
- To implement internationally accepted toxicity tests as part of the South African regulatory process, it is suggested that the following actions be taken.
 - An in-depth analysis and classification of the numerous toxicity test procedures and methods to facilitate practical application in the various fields of water use and discharge. With the implementation and revision of the water quality guidelines, which should be site and situation specific, a tentative guideline for toxicity should be included.
 - At the same time the DEEEP document must be revised. A follow-up WRC project can address the revision of the above guidelines.
 - > These guidelines should also address risk determination, i.e. the probability that the chronic effect should not exceed a certain level.
- It is recommended that the above toxicity tests be incorporated into the formulation of legislation and policy directives.
- It is recommended that compliance limits be designed and implemented to ensure efficient monitoring and enforcement.
- The development of a chronic toxicity test for water (ecological category "Fair and Good") testing should be addressed in a future WRC project. Because of difficulties currently experienced with the execution of the chronic Daphnia test described in the DEEEP document, this test was not included in the Methods Document, Volume 2. "Guideline for the Accreditation of Routine Aquatic Toxicity Testing Laboratories".

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APPENDIX A: TOXICITY TESTING LABORATORY SURVEY

COMPANY DETAILS
Company/testing laboratory name:
Department:
Contact person (name and designation):
Contact details:
Physical address of laboratory:
Postal address of laboratory:
Province:
Telephone number:
Fax number:
Cell number:
E-mail:
TOXICITY TESTS
Are toxicity tests performed in-house or subcontracted:
In-house ☐ Subcontracted ☐
List the toxicity tests performed at your laboratory/subcontracted laboratory and indicate if the laboratory is accredited for any of these methods:

	Method title/name		ich international	Accredited (√)
		method or kits (e.g	oratory developed LUS EPA, 2002)	
		incured or time (erg	<u>,, ee =, , ,, ====, </u>	
	Method title/name	Based on wh	ich international	Accredited (√)
			oratory developed	
		method or kits (e.g	J. US EPA, 2002)	
If not acc	redited for the above-mentioned tes	ts, is the laboratory	interested in futur	e accreditation of the
	and when?	•		
Does the	laboratory use in-house or commercia	I cultures?		
In-house	☐ Commercial cultures/kits ☐			
How man	y toxicity samples are analysed per m	onth?		
	0-10 11-20	20-50	50-100	100+
How man	y samples does the laboratory have to	perform to be finan	cially viable?	
	0-5 6-10	11-20	21-50	50+
PROFICII	ENCY TESTING SCHEMES			
Do you pa	articipate in any Proficiency Testing Sc	chemes?		
Yes 🗌	No 🗆			
	-			

If no, give a reas	son.
If yes, list the na	me of these Proficiency Schemes as well as the organisers.
Is there a need	for any new Proficiency Testing Schemes? List the method names.
RESEARCH	
Comments on p	ossible new research.
Would you like t	o participate in new research initiatives?
Yes	No 🗆
CUSTOMERS	
Broadly categor	se your customer base by indicating a percentage next to the list below:
Industry Municipal Water Boards Government Research Institu	tions
Do you link any	consultation to toxicity tests?
Yes 🗌	No 🗆

STAFF
How many staff members are directly involved in toxicity analyses?
List their designations, such as Client Coordinator, Sampler, Analyst, consultant, Laboratory Manager; etc.
Make a list of toxicity related training required by the laboratory.
Would the laboratory be interested in assistance or communication from more experienced/accredited laboratories?
Yes No No
In what format would this communication be preferred?
Meetings □
Training session's □
Aquatox Forum workshops/seminars □
Electronic format, such as e-mails □ Other □
Comments:
AQUATOX FORUM
Is any of your staff members an Aquatox Forum member?
Yes No No

would you	like to become and Aquatox Forum member?		
Yes 🗌	No 🗆		
Do you g webpage.	ve permission for the Aquatox Forum to link its Laboratory networkin	g page to your c	ompany
Yes 🗌	No 🗆		
Provide th	e webpage address for such a link.		
Would you	like to be part of future networking initiatives organised by the Aquatox F	Forum?	
Yes 🗌	No 🗆		
List any sı	nggestions for such networking initiatives.		
IMPLEME	NTATION OF ROUTINE TOXICITY TESTS FOR DWAF		
Does the I	aboratory have a copy of the DEEEP, 2004 document?		
Yes 🗌	No 🗆		
Does the I	aboratory do any tests for DEEEP use/effluent testing for license applicat	ions?	
Yes 🗌	No 🗆		
Tick the te	sts performed for DEEEP.		
	Test	In use	
	Oxygen demand BOD/COD		
	Acute toxicity		
	Vibrio fischeri luminescent bacterial test		
	Algal growth inhibition test		
	Daphnia acute toxicity test		
	Fish acute toxicity test		
	Chronic toxicity test		
	Invertebrates		
	Mutagenicity		
	Ames test		

Bioaccumulation potential

HPLC estimation

	being monitored with DEEE es into: stormwater systems, r		cations in the laboratory, (e	.g. industrial
Where ar	e the results being stored?			
	three biggest obstacles in ntation of toxicity testing can of Need for standardised toxici	occur e.g.:	to be resolved before the	e successful
1.1.2	Accreditation of laboratories	•		
1.1.3	Human resources.			
1.1.4 1.1.5	Sampling.	onartina		
1.1.6	Consultation/interpretation/re Statistical handling.	eporting.		
1.1.7	Equipment.			
1.1.8	Funding.			
1.1.9	Time constraints (e.g. samp	ling).		
1.1.10	Distances travelled to labora	•		
1.1.11	,	e, sample handling/storage/tr	• '	
1.1.12		and who is responsible for p	-	
1.1.13 1.1.14	Other	for testing with commercial la	adoratories.	
1				
2				
3				
Perceptio	on of toxicity testing as manag	gement tool in water resource	es management.	
	Excellent	Good	Poor	
C	Comments:			
_				

Current state of toxicity testing pertaining to water resources in South Africa.

- Status quo in water management areas.
- Overall status quo in region

	Excellent	Good	Poor
Co	omments:		

APPENDIX B: CONTACT DETAILS OF RESPONDENTS TO LABORATORY SURVEY

=	NaickerD@arc.agric.za	mtaylor@buckman.com	zarinas@mweb.co.za	carelsenc@dwaf.gov.za	pieter@ecomonitor.co.za	dewald@metsi.co.za	alisonc@erwat.co.za
E-mail	Naiç		zarina	carel		dewa	alisor
Contact No	Tel: 012 529 9260 Fax: 012 529 9258	Tel: 031 736 8800 Fax: 031 736 1593	Tel: 076 127 3013 Fax: 086 520 1390	Tel: 012 808 9500 Fax: 012 808 0338	Tel: 011 972 5298 Fax: 086 512 9528	Tel: 012 993 4671 Fax: 086 601 6927 073 163 4302	Tel: 011 929 7014
Postal address	Toxicology Private Bag X05 Onderstepoort 0110	P.O. Box 591 Hammarsdale 3700	P O Box 5742 Lenasia 1820	Department Water Affairs and Environment Private bag X313 Pretoria	PO Box 13434 Norkem Park Kempton Park 1631	P O Box 99462 Garsfontein East 0060	PO Box 13106
Physical address	Old Soutpan road Onderstepoort 0110	1 Buckman Boulevard Hammarsdale 3700	Santos Road Cooke Plant Randfontein 1759	Department Water Affairs and Environment Kwa-Mwanga road Roodeplaat	35 Pretorius Street van Riebeeck Park Kempton Park 1631	Randfontein Wastewater Treatment Works	Bapsfontein Road Kempton
Contact person	Dr Dharma Naicker	Michelle Taylor	Zarina Suliman Ahmed	Chris Carelsen	Pieter van Eeden	Dewald van Staden	Alison Chapman
Company / testing Iaboratory name	ARC-OVI Toxicology	Buckman Laboratories Microbiology	DD Science, Laboratory	Department: Water Affairs and Environment Resource Quality Services Analytical Services	EcoMonitor	Enviro Metsi (Pty) Ltd	ERWAT Laboratory

Company / testing Iaboratory name	Contact person	Physical address	Postal address	Contact No	E-mail
		1631	1631		
eThekwini Municipality Scientific Services Laboratory	Sibongile Maqubela	3 Halifax Rd New Germany 4000	PO Box 1038 Durban 4000	Tel: 031 311 8006 Fax: 031 311 8003	Sibongma@dmws.durban.gov.za
Golder Associates Research Laboratory Aquatic Toxicology Division	Bridget Shaddock	25 Main Avenue Florida 1709	PO Box 6001 Halfway House 1685	Tel: 011 672 0666 Fax: 011 672 0008	bshaddock@golder.co.za
Rand Water Hydrobiology	Elmari Krüger	Analytical Services 2 Barrage Road Vereeniging 1939	PO Box 3526 Vereeniging 1935	Tel: 016 430 8825 Fax: 016 455 2055	edekock@randwater.co.za
Renaissance Environmental Hub	Alétia Chapman	51 Waterson Street Sasolburg 1947	PO Box 2889 Sasolburg 1947	Tel: 016 973 3014 Fax: 086 663 4871	aletia.chapman@vodamail.co.za
SASOL Technology, R & D, Environmental Science and Technology, Sasolburg	Randal Albertus	1 Klasie Havenga Rd Sasolburg 1947	PO Box 1 Sasolburg 1947	Tel: 016 960 4838 Fax: 011 522 1808	Randal.albertus@sasol.com
ToxSolutions, Kits and Services cc	Hesmarie Pearson	12 Lily Road Brackenhurst ext 2 1448	12 Lily Road Brackenhurst ext 2 1448	Tel: 011 867 1274 Fax: 088 011 867 1274	Hesmarie@telkomsa.net
Unilever Centre for Environmental Water Quality	Dr Nikite Muller	Old Geology Building Artillery Road Rhodes University	UCEWQ IWR Rhodes University PO Box 94	Tel: 046 603 8532 Fax: 046 622 9427	N.muller@ru.ac.za

Contact No E-mail		Tel: 033 341 1342 <u>ian.bailey@umgeni.co.za</u> Fax: 033 341 1501 <u>kathy.milford@umgeni.co.za</u>	Tel: 011 559 2951 <u>econ@ui.ac.za</u> Fax: 011 559 2286 alternatively <u>ycloete@uj.ac.za</u>	II Tel: 012 529 8031 <u>annette.venter@up.ac.za</u> Fax: 012 529 8304	псе
Postal address	Grahamstown 6140	Umgeni Water PO Box 9 Pietermaritzburg 3200	Department of Zoology PO Box 524 Auckland Park 2006	Department of Paraclinical Sciences Section: Pharmacology & Toxicology	Faculty of Veterinary Science University of Pretoria Private Bag X04 Onderstepoort 0110
Physical address	Grahamstown 6140	310 Burger Street Pietermaritzburg	D3Lab334 Department of Zoology University of Johannesburg APK Campus Kingsway Auckland Park 2006	Paraclinical Sciences Pharmacology & Toxicology Faculty of Veterinary	Science Soutpan Rd Onderstepoort 0110
Contact person		lan Bailey	Yolandi Cloete	Annette Venter	
Company / testing laboratory name	Institute for Water Research Rhodes University	Umgeni Water Hydrobiology	Econ@UJ	University of Pretoria Department: Paraclinical Sciences: Pharmacology & Toxicology	

APPENDIX C: QUESTIONS FOR DWAF & ACADEMIC INSTITUTIONS

- 1. Can toxicity testing be used as a water quality management tool?
- 2. State of routine toxicity testing within the DWAF
- 3. What is needed for the routine implement of toxicity testing, nationwide?
 - a. Development of toxicity standards
 - b. Promulgation of such standards in terms of the NWA
 - c. Development of licence conditions
 - d. Call for toxicity testing in licences
 - e. Comparison of results against a set of standards
 - f. Compliance conditions
 - g. Legal action do the stakeholders expect legal action to be taken for noncompliance
- 4. What are the capacity problems associated with implementation of routine toxicity testing?
 - a. Funding
 - b. Human Resource component
 - c. Infrastructure?
 - i. Laboratories (DWAF/Private) what is the role they should play?
 - 1. Accreditation/GLP status sufficient for regulatory testing?
 - 2. Who oversees national laboratory accreditation (DWAF/SANAS)?
 - 3. Can Resource Quality Services (RQS) laboratory serve as core-service laboratory?

Action/working groups that focus on quality management – what are the requirements? Future workshop between DWAF regions, National DWAF head office and laboratories – what are your comments with respect to the relevancy of such a meeting taking place?

APPENDIX D: DWAF PERSONNEL INTERVIEWED USING APPENDIX C

The following is an alphabetical list of the DWAF personnel interviewed (email/telephonically/in person) with regards to the questions posed in Appendix C.

Name	Designation	DWAF Directorate	
Ms S. Bohlmer	Scientist	Resource Quality Services	
Mr G. Cilliers	Assistant Director	Resource Quality Services	
Mr L. Gravelot-Blondin	Deputy Director	Water Quality Management KwaZulu-Natal	
Mr P. Herbst	Deputy Director	Water Use Efficiency	
Dr S. Jooste	Principal Specialist Scientist	Resource Quality Services	
Mr M. Keet	Deputy Director	Water Quality Management Gauteng South	
Ms W. Kloppers	Deputy Director	Water Quality Management Western Cape	
Mr A. Lucas	Deputy Director	Water Quality Management Eastern Cape	
Mr J. Streit	Deputy Director	Water Quality Management Northern Cape	
Dr J. van der Merwe	Deputy Director	Water Quality Management Free State	
Mr P. Venter	Deputy Director	Water Quality Management North West and Gauteng North	

APPENDIX E: ACADEMICS INTERVIEWED USING APPENDIX C

The following is an alphabetical list of university/technikon personnel interviewed (email/telephonically/in person) with regards to the questions posed in Appendix C.

Name	Designation	Organisation
Dr J. Odendaal	Lecturer	Cape Peninsula University of Technology
Dr E. Pool	Lecturer	University of the Western Cape
Prof A. Reinecke	Lecturer	University of Stellenbosch
Dr P. Stegman	Lecturer	Vaal University of Technology
Dr R. Snyman	Lecturer	Cape Peninsula University of Technology
Prof H. van Wyk	Lecturer	University of Stellenbosch

APPENDIX F: LICENCE CONDITIONS EXAMPLES

A suitably qualified person must be appointed to direct the sampling and access and interpret the results for reporting. The water from the final discharge point(s) should be tested for toxicity. The following tests must be conducted:

Acute Toxicity Tests

todio Toxiony Toolo		
Tests	Test Frequency	Limits
Daphnia pulex/magna	Fortnightly	See RCS ¹
Poecilia reticulate / Danio rerio	Fortnightly	See RCS ¹
Vibrio fischeri	Weekly	See RCS ¹
Selanastrum capricornutum	Weekly	See RCS ¹

¹Resource Classification Specifications

Water Resource Classification System

Trator Recourse Glacomoution Gyotom		
Ecological Category	Criteria	Toxicity Observed
Natural	No toxicity of any kind	None
Fair & Good	No lethality (short or long term)	Sub-lethal
Poor		Lethality

Acute Toxicity Exhibited by Individual Toxicity Tests

Acute toxicity units	Description ²	Colour Code	Ecological Category
>100 Tu _a	Highly acutely toxic		Poor
10-100 Tu _a	Acutely toxic		P001
2-10 Tu _a	Mildly acutely toxic		Fair & Good
1-2 Tu _a	Negligently acutely toxic		Fall & Good
<1 Tu _a	Not acutely toxic		Natural

²⁽The Netherlands RIZA system, DWAF, 2003

- It is expected that the applicable ecological class for work-horse river systems would be that of Fair/Good and subsequently sub-lethal toxicity would be observed
- It is not expected that discharge would be allowed into a Natural Ecological Category
- The Poor Ecological Category is not a management class and as such is not expected that such a category could/would be sustained

Ecological Category	Description (on the basis of the highest acute toxicity unit (TUa) found in the battery of toxicity definitive tests performed) ³
Natural	No Acute Hazard. None of the tests shows a toxic effect
Fair	Slight Acute Hazard. The percentage effect observed in at least 1 toxicity test is significantly higher than in the control, but the effect level is below 50% (TU _a is <1)
Good	Acute hazard. The LC/LE ₅₀ is reached or exceeded in at least one test, but in the 10 fold dilution of the sample the effect level is below 50% (TU _a is between 1 and 10).
Poor	High acute hazard . The LC/LE $_{50}$ is reached in the 10 fold dilution for at least one test, but not in the 100 fold dilution (TU $_a$ is between 10 and 100).
Poor	Very high acute hazard . The LC/LE ₅₀ is reached in the 100 fold dilution for at least one test (TU_a is >100).

³Personal communication, 2009

If toxicity limits are exceeded:

- 1. Re-test (and check against chemical limits needs refinement)
- 2. Period to correct discharge
- 3. Fine/Imprisonment

Toxicity tests must be performed against standard methods Reference toxicity test results for the accredited laboratory must be made available on request