Development of research support to enable the issuing of aquatic toxicity-based water use licenses:

Guideline document

Report to the
Water Research Commission

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EXECUTIVE SUMMARY

In order to reduce and prevent the degradation of water resources as well as assess its quality, the South African National Water Act (Act 36 of 1998) (NWA) mandated that a national monitoring system should be established. A fundamental premise of the Act is that an ecological effect-based approach needs to be applied to water resource management, supported by the regular aquatic bioassay testing of water resources and complex industrial wastewaters (effluents) which are being released into water resources. To comply with requirements of the NWA, the National Toxicity Monitoring Program (NTMP) for water resource management and the Direct Estimation of the Ecological Effect Potential Approach (DEEEP), to manage effluent discharge into surface waters, was designed. The incorporation of the required toxicity bioassays forms part of Integrated Wastewater Management Plan implementation as well as Water Use License approvals. However, the correct use of these bioassay requirements and how they are included into license conditions has been hampered by non site-specific wordings resulting in ambiguity and therefore the exclusion of these conditions by personnel due to lack of available information. This document was compiled to provide background information on the various approaches applied internationally, both for management approaches as well as toxicity assessments, as well as information in the form of a guideline document outlining the use of the Integrated Water Use Authorisation Bioassay Toolkit.

Literature studies indicate that toxicity tests are widely applied internationally by water and wastewater management authorities. Whilst numerous approaches exist as resource management approaches, internationally the use of bioassays to assess the overall ecological status in water resources remains a common denominator. These bioassays are used to compliment the standard evaluation tools such as chemical analysis and biomonitoring, enabling observed in field effects to be interpreted against the integrated laboratory exposures in relation to the measured chemical concentrations. The use of bioassays has been shown to save both time and money when identifying areas of concern, prioritising sites for remediation, increased monitoring or improved treatment (improved technology) approaches.

The Integrated Water Use Authorisation Bioassay Toolkit has been designed with Water Use License Authorisations in mind (both new and existing applications). However, the toolkit can additionally provide method information to guide both clients and consultants in the bioassay requirements needed to comply with the Water Use License conditions. The developed toolkit has been developed for use by the Government institutions which are the custodians of water resources (e.g. the Department of Water and Sanitation and the Department of Environment Affairs), Licensees, Consultants and Toxicity Testing Laboratories.

Workshops were conducted with various stakeholders in order to identify the frustrations and the needs of the end users. This was then translated into the input and output requirements of the toolkit as well as additional information which would make the adoption of this approach more widespread. For this reason the information provided in Chapter 1 and 2 is geared to increase the understanding of “why” conducted toxicity bioassay tests, whilst Chapter 3 fills the capacity gap with easy instructions on “how” to use the toolkit, as well as how to interpret and implement the license conditions generated in the output. Information on quality requirements, additional international standards, the current Water Use License Application checklist and how Hazard Classifications are calculated has been included in the Appendices. Whilst this information does not have a direct impact on the Integrated Water Use Authorisation Bioassay Toolkit, the additional information provided in these sections will assist the end user to apply Water Use License conditions, ensuring traceability of results to protect clients, compliance requirements and gather sufficient information for license renewals.

It is recommended that training on both the toolkit and guideline document should be treated as a priority. Currently a follow-up project has been awarded to assess industry specific criteria and provide additional information to refine the toolkit. The inclusion of methods and criteria to assess estuary and marine environments as well as human health should be addressed with follow-up projects. Additionally methods which include the evaluation of sediment and solid waste should be researched and developed for inclusion into Water License conditions. The potential for the toolkit to identify mitigation approaches should emergency spills take place is also an area which needs clarification.

During the workshops, positive feedback was received regarding the need and application of the toolkit as well as questions regarding the inclusion of the information gathered and its applicability for various other interlinked processes such as Resource Quality Objectives and the national databases. The toolkit was therefore developed with expansion into these areas in mind. Feedback and suggestions from users will be encouraged to streamline further needs. Further funding will be required in order to continue the toolkit development allowing it to fulfil a vital role in driving compliance monitoring, quality objectives (e.g. Green Drop compliance), easy to understand/implement license conditions and the successful maintenance of aquatic resources within South Africa.

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ACRONYMS & ABBREVIATIONS

ANZECC	Australian and New Zealand Environment and Conservation Council
ARMCANZ	Agriculture and Resources Management Council of Australia and New Zealand
ASTM	American Society for Testing and Materials
BAT	Best Available Technology
BATEA	Best Available Technology Economically Achievable
BCF	Bio-concentration Factor
BEP	Best Environmental Practice
BOD	Biological Oxygen Demand
BPG	Best Practice Guideline
CAEAL	Canadian Association of Environmental Analytical Laboratories
CEPA	Canadian Environmental Protection Act
CID	Characterisation of Industrial Discharges
CMA	Catchment Management Agency
COD	Chemical Oxygen Demand
CSIR	Council for Scientific and Industrial Research
CWA	Clean Water Act
DEEEP	Direct Estimation of Ecological Effect Potential
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DTA	Direct Toxicity Assessment
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
EC	Effective Concentration
EC50	Median Effective Concentration
ECRW	Effluent Concentration in Receiving Waters
EEC	European Economic Community
EIA	Environmental Impact Assessment
EPA	Environmental Protection Agency
EPS	Environmental Protection Series
EU	European Union
GLP	Good Laboratory Practice
HOCNF	Harmonised Offshore Chemical Notification Format

ICE	Integrating Controlling of Effluents
ICM	Integrated Catchment Management
ISO	International Organization for Standardization
ISTA	International Symposium on Toxicity Assessment
IWWMP	Integrated Water and Waste Management Plan
LC	Lethal Concentration
LC50	Median lethal concentration
LID	Lowest Ineffective Dilution
LOEC	Lowest Observed Effect Concentration
MISA	Municipal and Industrial Strategy for Abatement
NLA	National Laboratory Association
NOEC	No Observable Effect Concentration
NOECa	Acute No Effect Concentration
NOECc	Chronic No Effect Concentration
NPDES	National Pollutant Discharge Elimination System
NTMP	National Toxicity Monitoring Programme
NWA	National Water Act
NWQMS	National Water Quality Monitoring Strategy
OECD	Organisation for Economic Cooperation and Development
PEC	Predicted Environmental Concentrations
PNEC	Predicted No-Effect-Concentration
PPP	Polluter Pays Principle
PTS	Proficiency Testing Scheme
QA	Quality Assurance
QC	Quality Control
RDM	Resource-Directed Measures
RIZA	Netherlands' Institute for Inland Water Management and Wastewater Treatment
RQO	Resource Quality Objective
RQS	Resource Quality Services
SA NWA	South African National Water Act, 1998 (Act 36 of 1998)
SABS	South African Bureau of Standards
SANAS	South African National Accreditation System
SANS	South African National Standard
SC	Sub Committee
SDC	Source-Directed Controls
SETAC	Society of Environmental Toxicology and Chemistry

TC	Technical Committee
TEF	Toxicity Emission Factor
TIE	Toxicity Identification and Evaluation
TRE	Toxicity Reduction Evaluation
TU	Toxicity Unit
UK	United Kingdom
US EPA	United States Environmental Protection Agency
USA	United States of America
WEA	Whole Effluent Assessment
WET	Whole effluent toxicity
WETT	Whole Effluent Toxicity Tests
WFD	Water Framework Directive
WRC	Water Research Commission
WUL	Water Use Licences
WULA	Water Use Licence Application

GLOSSARY

Acute
Denotes effect within a short period of time (seconds, minutes, hours, or a few days) in relation to the life span of the test organism.

Acute toxicity
Rapid adverse effect (e.g. death) caused by a substance in a living organism. Can be used to define either the exposure or the response to an exposure (effect).

Acute-chronic ratio
The species mean acute value divided by the chronic value for the same species.

Additive toxicity
The toxicity of a mixture of chemicals that is approximately equivalent to that expected from a simple summation of the known toxicities of the individual chemicals present in the mixture (i.e. algebraic summation of effects).

Antagonism
A phenomenon in which the effect or toxicity of a mixture of chemicals is less than that which would be expected from a simple summation of the effects or toxicities of the individual chemicals present in the mixture (i.e. algebraic subtraction of effects).

Aquatic ecosystem
Any water environment from small to large, from pond to ocean, in which plants and animals interact with the chemical and physical features of the environment.

Bioaccumulation
General term describing a process by which chemical substances are accumulated by aquatic organisms from water, either directly or through consumption of food containing the chemicals.

Bioassay
A test that exposes living organisms to several levels of a substance that is under investigation, and evaluates the organisms' responses.

Bio-available
The fraction of the total of a chemical in the surrounding environment that can be taken up by organisms. The environment may include water, sediment, soil, suspended particles, and food items.

Biochemical (or biological) oxygen demand
The decrease in oxygen content in mg/L of a sample of water in the dark at a certain temperature over a certain of period of time which is brought about by the bacterial breakdown of organic matter. Usually the decomposition has proceeded so far after 20 days that no further change occurs. The oxygen demand is measured after 5 days (BOD5), at which time 70% of the final value has usually been reached.

Biological assessment
Use and measurement of the biota to monitor and assess the ecological health of an ecosystem.

Catchment
The total area draining into a river, reservoir, or other body of water.

Chronic
Lingering or continuing for a long time; often for periods from several weeks to years. Can be used to define either the exposure of an aquatic species or its response to an exposure (effect). Chronic exposure typically includes a biological response of relatively slow progress and long continuance, often affecting a life stage. Means occurring in a relatively long period of exposure, usually a substantial portion of the life span of the organism (such as 10% or more).

Direct toxicity assessment (DTA)
The use of toxicity tests to determine the acute and/or chronic toxicity of wastewater discharges or total pollutant loads in receiving waters. (Assesses the toxicity of mixtures of chemicals rather than individual chemicals).

EC_{50} (median effective concentration)
The concentration of material in water that is estimated to be effective in producing some lethal response in 50% of the test organisms. The LC_{50} is usually expressed as a time-dependent value (e.g. 24-hour or 96-hour LC_{50}).

Effluent
A complex waste material (e.g. liquid industrial discharge or sewage) that may be discharged into the environment.

End-points
Measured attainment response, typically applied to ecotoxicity or management goals. Is the statistic that is estimated as the result of a test.

Eutrophic
Abundant in nutrients and having high rates of productivity frequently resulting in oxygen depletion below the surface layer of a water body.

Guideline trigger values
These are the concentrations (or loads) of the key performance indicators measured for the ecosystem, below which there exists a low risk that adverse biological (ecological) effects will occur. They indicate a risk of impact if exceeded and should 'trigger' some action, either further ecosystem specific investigations or implementation of management/remedial actions.

LC_{50} (median lethal concentration)
The concentration of material in water that is estimated to be lethal to 50% of the test organisms. The LC_{50} is usually expressed as a time-dependent value, e.g. 24-hour or 96-hour LC_{50}, the concentration estimated to be lethal to 50% of the test organisms after 24 or 96 hours of exposure.

Lethal
Causing death by direct action. Death of aquatic organisms is the cessation of all visible signs of biological activity.

LOEC (Lowest observed effect concentration)
The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls. When derived from a life-cycle or partial life-cycle test, it is numerically the same as the upper limit of the MATC.

MATC (Maximum acceptable toxicant concentration)
The maximum concentration of a toxic substance that a receiving water may contain without causing significant harm to its productivity or uses as determined by chronic toxicity tests.

NOEC (No observed effect concentration)
The highest concentration of a toxicant at which no statistically significant effect is observable, compared to the controls; the statistical significance is measured at the 95% confidence level.

Organism
Any living animal or plant; anything capable of carrying on life processes.

Pollution
The introduction of unwanted components into waters, air or soil, usually as result of human activity; e.g. hot water in rivers, sewage in the sea, oil on land.

Quality assurance (QA)
The implementation of checks on the success of quality control (e.g. replicate samples, analysis of samples of known concentration).

Quality control (QC)
The implementation of procedures to maximise the integrity of monitoring data (e.g. cleaning procedures, contamination avoidance, sample preservation methods).

Sediment
Unconsolidated mineral and organic particulate material that settles to the bottom of aquatic environment.

Species
A group of organisms that resemble each other to a greater degree than members of other groups and that form a reproductively isolated group that will not produce viable offspring if bred with members of another group.

Sub-lethal
Involving a stimulus below the level that causes death. Detrimental to the organism but below the level which directly causes death within an exposure period.

Toxicant
A chemical capable of producing an adverse response (effect) in a biological system at concentrations that might be encountered in the environment, seriously injuring structure or function or producing death. Examples include pesticides, heavy metals and bio-toxins (i.e. domoic acid, cigua-toxin and saxi-toxins).

Toxicity
The inherent potential or capacity of a material to cause adverse effects in a living organism

Toxicity identification and evaluation (TIE)
Toxicity characterisation procedures involving use of selective chemical manipulations or separations and analyses coupled with toxicity testing to identify specific classes of chemicals and ultimately individual chemicals that are responsible for the toxicity observed in a particular sample.

Toxicity test
The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical).

Whole effluent toxicity testing
The use of toxicity tests to determine the acute and/or chronic toxicity of effluents.

SYNONYMS

Biological test guideline/protocol/procedure;
Toxicity test/ecological bioassays
Biological test guideline/test method/test procedure/test protocol;
Biological test standard/reference method/standardized toxicity test

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

1.1.1 RELEVANCE

Water is a strategic component in the social- and economic development initiatives of South Africa and a constitutional imperative in respect of human well-being and the environment. The desk study was undertaken to facilitate the use of whole effluent aquatic toxicity bio-assays as an instrument to enhance water quality by:

- ***Determining*** the status quo of the international application of aquatic toxicity testing in compliance monitoring/legislation;
- ***Benchmarking*** the above with the situation in South Africa;
- ***Facilitating the alignment of South African legislation/policy*** with international trends in the field of licence and compliance monitoring; and to
- ***Assist with*** the application of aquatic bio-assays by developing and benchmarking a decision making support system enabling the granting of toxicity based water use licences.

Legislative processes are time consuming and intricate as policy formulation and prescriptions have to satisfy the expectations of diverse groups such as affected citizens, activists, researchers, legislators and those managers and agencies or departments responsible for the implementation thereof. The desk study was in essence aimed at determining whether South Africa is currently in a position to formalize and implement measures aimed at enhancing viable and quality water resources as envisaged in the National Water Act (RSA, 1998). It must be noted that toxicity testing is but one of the instruments to achieve the goals set out in the act.

1.1.2 DEFINITION

Bioassays or toxicity tests describe standardized experiments that determine the toxicity of a substance or material by evaluating its effect on living organisms. Tests are designed to use appropriate organisms and sensitive effect measurements in the media of interest for a specified test duration. The tests are conducted as a means of establishing a causal relationship between the biological effect and the substance or material being tested. Biological test methods (BTMs) or toxicity tests are developed to evaluate the concentrations of a material or substance and duration of exposure that is required to produce an effect (e.g., dead or alive) or a degree of effect (e.g., 25% inhibition). The substance to be tested can be a specific chemical or chemical product, or the material under evaluation can be effluent, leachate, elutriate, or contaminated surface water, soil, or sediment. Tests can be conducted on microorganisms, vertebrates, invertebrates, and plants species and examine effects on survival, growth, reproduction, behaviour, bioaccumulation, activity, or other biological markers. Tests can be conducted in water, soil, or sediment, and the duration of exposure is typically dictated by the effect of interest, that is, partial to full life cycle, or multi-generational (Taylor and Scroggins, 2013).

1.1.3 BASIC PRINCIPLES

The basic principle of whole effluent aquatic toxicity bio-assays is not complicated: Aquatic and terrestrial test organisms are subjected to whole effluent water samples to determine the effect thereof on biological parameters such as survival, growth, mobility or reproduction. In this manner the toxicity of the whole sample, including unknown substances and the combined effects, can be investigated. These tests are more often referred to as "biological assays" or bio-assays. There are numerous bio-assays, each with different end-points, durations and species. These tests can be performed with single species or with simple communities (multiple species) under laboratory conditions, or *in situ* e.g. caged studies or artificial streams (Clements and Kiffney, 1996; Walker et al., 2001). Due to the complexity of the latter this document will mainly focus on single species tests under laboratory conditions using standardised methods.

The selection criteria of the test organisms include ecological relevance, easy and cost efficient maintenance and whether the behaviour, life cycle and habitat requirements of the organisms are known. It is widely recommended that a battery of bioassays are conducted with several (preferably 3-5) different organisms on different trophic levels/functional groups (e.g., bacteria, phytoplankton, invertebrates, fish and plants) (Johnson et al., 2004; Diaz-Baez and Dutka, 2005). There are several reasons for this. Firstly, there is variation between the sensitivity of the different species, and therefore using several species increases the reliability of the tests interpretation. Secondly, no single species can indicate all the substantial end-points (Johnson et al., 2004). This is a way to obtain a better view of the effects on ecosystem functioning and on different trophic levels (COHIBA, 2010).

1.1.4 VALUE OF BIOLOGICAL ASSESSMENT METHODS

At present the control of hazardous substances in effluents/wastewater is mainly based on traditional chemical analysis and setting concentration limits on individual substances or substance groups. A prerequisite for management based on chemical approach is that the controlled substances are known and can be identified from the effluent. Most effluents are complex mixtures consisting of numerous chemical substances, all of which are not known. The chemical approach is therefore insufficient when managing complex wastewaters, despite its effectiveness in dealing with simple effluents (effluents consisting of a few, known substances). The problem with policy instruments based on the chemical approach is that they disregard discharges of many potentially hazardous substances and do not address the possibility of combined effects on the environment. Banning or reducing the concentration of one hazardous substance does not always prevent it from being replaced by another equally hazardous substitute or with a substitute carrying unknown properties (COHIBA, 2010).

The sheer number of existing commercially-used chemicals, not to mention their metabolites and unintended by-products created in the manufacturing processes, makes it difficult to address the pollution problem by means of monitoring and limiting concentrations of individual substances. It is an impossible task to identify all the chemicals and their metabolites from the effluent and to assess the combined effects of the countless possible mixtures of these chemicals. Bio-assays make it possible to evaluate the toxicity of the effluent as a whole, thus also taking into account the combined effects and the effects of unknown substances. For example it enables setting a whole effluent toxicity limit based on the biological effects of the effluent. Biological assessment methods supplement the chemical analyses, and they are valuable tools for early warning of the potential hazard of wastewater effluents (COHIBA, 2010).

One of the advantages of bio-assays is that they provide a more comprehensive picture of the effluent's environmental effects than the chemical concentration data. It can be seen as a "link between chemistry and ecology" as it directly indicates the effects of an effluent on the survival, growth and/or reproduction of organisms (Wharfe, 2004). Chemical analysis based on extracting and determining concentrations of individual chemicals does not adequately simulate the situation where living cells/organisms are exposed to

the whole effluent. All the substances present in the effluent affect the metabolism of test organisms to some extent. In toxicity testing this is taken into account, which includes the unknown substances and synergistic effects. In addition, many important biological processes (such as bioavailability and bioaccumulation, selective intake in a cell and xeno-biotic metabolism, etc.) are inherent facets of these tests as since living cells/organisms are used (Wharfe et al., 2004; Wharfe, 2004; COHIBA 2010).

Bio-assays have multiple value and application:

- It is a cost effective way to screen effluents and detect those effluents that have significant adverse effects on aquatic organisms (Chapman, 2000, Johnson et al., 2004). It is an instrument to identify effluents which need urgent toxicity reduction actions. Acute tests are especially suitable for this purpose, as large numbers of effluent samples can be screened and ranked cost-efficiently in a limited time period. (There are hazardous substances, however, which are present in such small concentrations that acute effects might not occur.);
- Chronic test reflect the characteristics and long term effects of effluents more thoroughly. It should be borne in mind that potentially chronic and sub-lethal effects can be present in the effluent even if there are no signs of acute toxicity (OSPAR, 2007). The control and reduction of wastewater toxicity might not be sufficient if based solely on acute toxicity (Wharfe et al., 2004; COHIBA 2010);
- Whole Effluent Assessment (WEA) can also be used in "toxicity tracking" i.e. to identify the source of toxicity by conducting these tests upstream in the wastewater sewage system or upstream of an industrial process (Hutchings et al., 2004; Tinsley et al., 2004);
- Following initial WEA screening and/or characterization it is possible to conduct Toxicity Identification Evaluation (TIE) and Toxicity Reduction Evaluation (TRE) (Hutchings et al., 2004). The aim of TIE is to identify the substances or wastewater fractions responsible for the effluent's toxicity. The objective of TRE is to identify the source of these substances and to plan adequate reduction measures for the substances in question (COHIBA, 2010). Bio-assays in combination with chemical analysis can be utilised where the exact substances causing the toxicity need to be identified (Hutchings et al., 2004) and it can reduce the costs of conducting these procedures.
- WEA can also be utilised in derivation of environmental/effluent quality targets, for example whole effluent toxicity limits can be set on wastewaters. These limits can be sector specific. If such limits are imposed on wastewater dischargers, the compliance monitoring should of course also include WEA tests;
- Data derived from toxicity tests have many practical applications, such as environmental risk assessments, regulatory compliance and enforcement, existing substance and new product testing (drugs, pesticides, herbicides, household products, etc.), routine monitoring required through environmental regulations, corporate decision making to understand the potential impact of manufacturing and treatment processes, contaminated site assessment and remediation applicability to disposal permitting for contaminated dredged sediments (Taylor and Scroggins, 2013)

As mentioned above, 3-5 different organisms are used in an initial WEA (e.g. screening or characterisation), but after this it is sometimes possible to proceed using only the species that has proven to be most sensitive to effluent in question. This is a way to increase the cost-effectiveness of the TRE procedure. The TRE and TIE procedures are commonly used in the United States of America but it has been concluded that these methods should be more fully developed before implementation (OSPAR, 2007; COHIBA, 2010).

Utilisation of WEA in hazardous substances management can be crudely divided into two approaches: a site-specific approach and hazard-based approach. The site-specific approach takes into account the dilution of effluent when it is discharges into the recipient water. The emphasis is on environmental or water quality targets. The disadvantage of this approach is that dilution does not reduce the actual amount of hazardous substances (only the concentration), which might cause problems in the long run when dealing with persistent and bio-accumulating substances. In the hazard-based approach the focus is on the load before

discharge (source control type of solution). A combination of these approaches is also possible (COHIBA, 2010).

1.1.5 LIMITATIONS OF BIOLOGICAL ASSESSMENTS

WEA approach also has inherent disadvantages and is the therefore not a perfect instrument for hazardous substances management (Chapman, 2000). The best way to control hazardous substances is to find a combination of tools that supplement each other (Pedersen et al., 1994). When implementing WEA based policy measures both benefits and limitations of WEA should be borne in mind (COHIBA, 2010).

The most important limitations of WEA are summarised below:

- WEA identifies potential risk and cannot be used in isolation to conduct an entire ecological risk assessment;
- It is not an instrument to identify the exact substances causing the adverse effects, but rather a step in the identification process;
- There is inherent variability in WEA (as is in all analytical methods). Factors accounting for the variability are intra- and interspecies variability as well as intra and inter-laboratory variability. Variability can be reduced by using standardised or validated methods.

The use of living organisms has been criticised for ethical reasons, especially in case of fish or other vertebrates (Wharfe et al., 2004). However, as there are no methods of similar sensitivity and of ecological relevance, such tests should be accepted. Living organisms have species specific requirements for the culture conditions, which may necessitate adjustment of the sample pH or salinity, for example. These actions may have an effect on the bioavailability or solubility of certain hazardous substances (COHIBA, 2010).

1.2 REVIEW: INTERNATIONAL INSTITUTIONS

Examining the nature and scope of bioassay related papers and reports on the agendas of prominent international conventions/agreements as well as for scientific meetings and symposia, proved to be most valuable in the assessment of the global *status quo* relating to toxicity testing. Evaluation of diverse knowledge bases by participants (scientists, managers and legislators), debating the merit and application of methodology and policy, and ultimate decision making /consensus at these forums facilitated the review and international application of measures to enhance water quality control and monitoring. Contributions and situational reports from some of the prominent forums which may enhance the objectives of this paper are summarised below. Particular attention was paid to those factors which can facilitate the South African decision making process.

1.2.1 EUROPEAN UNION (EU)

The Water Framework Directive (WFD) was circulated in 2000 with the aim 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 (Chapman et al., 2011a). It has been described as the most progressive piece of European legislation (Griffiths, 2002). In order to assess toxic impacts of contamination in the aquatic environment the use of acute toxicity tests are used in this framework (Chapman et al., 2011a).

Chapman et al. (2011a) found that toxicity testing of contaminants to protect human and aquatic health occurs in the EU under Commission Directives that directs the risk assessment of new substances, existing substances, and biocidal products (NRC, 2006). The Commission Directive 93/67/EEC outlines 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 towards fish.
- Acute toxicity towards daphnia.
- Algal growth inhibition.
- Bacterial growth inhibition.

The Directive 2000/60/EC of the European Parliament and of the Council (2000 was then amended in 2001, 2008 and 2009) provides a detailed overview of, and action plans to guide water resource management in the EU (Chapman et al., 2011a). Its value lies in the fact that it authoritatively promotes the application of scientific knowledge in practice, but more importantly ensures that policies and action plans are the end product of political debate and consensus by member nations. The document contains valuable data and expertise where water resource policy, and implementation thereof, is considered. The brief policy related excerpts below illustrate the value of the Directive in respect of guidance and uniform approach, and relates to the objectives set out in our own National Water Act (RSA, 1998):

"Community water policy requires a transparent, effective and coherent legislative framework. The Community should provide common principles and the overall framework for action. This Directive should provide for such a framework and coordinate and integrate, and, in a longer perspective, further develop the overall principles and structures for protection and sustainable use of water in the Community in accordance with the principles of subsidiarity".

"This Directive aims at maintaining and improving the aquatic environment in the Community. This purpose is primarily concerned with the quality of the waters concerned. Control of quantity is an ancillary element in securing good water quality and therefore measures on quantity, serving the objective of ensuring good quality, should also be established."

In deriving environmental quality standards for pollutants listed for the protection of aquatic biota, member States are required to act in accordance with the set provisions. Standards may also be set for water, sediment or biota and where possible, both acute and chronic data shall be obtained for the taxa which are relevant for the water body type concerned as well as any other aquatic taxa for which data are available (Chapman et al., 2011a). The 'base set' of taxa are:

- algae and/or macrophytes,
- daphnia or representative organisms for saline waters and
- fish.

The procedure to be applied for the setting of a maximum annual average concentration (quality standards) includes setting appropriate safety factors in each case consistent with the nature and quality of the available data and the guidance provided (relevant section of the Technical guidance document in support of Commission Directive 93/67/EEC on risk assessment for new notified substances, and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances) safety factors are set out below:

- At least one acute L(E)C_{50} from each of three trophic levels of the base set, factor 1 000.
- One chronic NOEC (either fish or daphnia or a representative organism for saline waters), factor 100.
- Two chronic NOECs from species representing two trophic levels (fish and/or daphnia or a representative organism for saline waters and/or algae).
- Chronic NOECs from at least three species (normally fish, daphnia or a representative organism for saline waters and algae) representing three trophic levels, factor 10 (Chapman et al., 2011a).

Other cases, including field data or model ecosystems, which allow for more precise safety factors to be calculated and applied include:

- where data on persistence and bio-accumulation are available, these shall be taken into account in deriving the final value of the environmental quality standard;
- the standard derived should thus be compared with evidence from field studies. Where anomalies appear, the derivation shall be reviewed to allow a more precise safety factor to be calculated;
- the standard derived shall be subject to peer review and public consultation including to allow a more precise safety factor to be calculated.

1.2.2 ORGANIZATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD)

The OECD has published a document promoting the use of biological tests for water pollution assessment and control in industrialized and developing countries (OECD, 1987). The guidance document suggests desirable approaches but does not specify what test methods are to be used or the toxicity levels at which certain cautionary actions should be taken. The OECD states that as a result of the differing needs and circumstances within member countries, determination of these matters must be left to the judgement of individual countries for complex effluents are founded. The OECD concluded that toxicity testing procedures must be based on a sound and generally accepted scientific basis. Evaluation of effluent toxicity, particularly when analysed in conjunction with engineering, chemical and ecological data, can provide a valid indication of the effects of toxic effluents on receiving systems, and can significantly improve the development of regulatory requirements to protect “aquatic life" (OECD, 1987).

Currently countries throughout the world are using OECD biological toxicity assays for water quality testing (Appendix B: list of published standards) (see OECD webpage http://www.oecd-ilibrary.org/content/package/chem_guide_pkg-en for list of published standards). Fish, water flea, algal tests and bacterial luminescent bioassays have been successfully standardised so far. Countries like the USA, Germany, and France have their own standard methods for biological toxicity tests for water. These closely resemble the standard methods of organizations such as the International Organisation for Standardisation (ISO), the Organization for Economic Cooperation and Development (OECD) and the American Society for Testing Materials (ASTM).

1.2.3 INTERNATIONAL ORGANIZATION FOR STANDARDISATION (ISO)

ISO is an independent, non-governmental membership organization and the world's largest developer of voluntary International Standards. Currently ISO has 165 member countries that are the respective national standards bodies around the world, with a Central Secretariat that is based in Geneva, Switzerland. The South African Bureau of Standards (SABS) represents South Africa at ISO and currently South Africa is voting and contributing as a P-member at ISO.

According to ISO, International Standards make things work. They provide world-class specifications for products, services and systems to ensure quality, safety and efficiency. They are instrumental in facilitating international trade. For business, they are strategic tools that reduce costs by minimizing waste and errors and increasing productivity. They assist companies to access new markets, level the playing field for developing countries and facilitate free and fair global trade.

ISO has published more than 19 500 International Standards covering almost every industry, from technology, to food safety, to agriculture and health care. Experts from all over the world develop the standards that are required by particular sectors. This means that these standards reflect a wealth of international experience and knowledge.

Water Quality is discussed under Technical Committee TC147 (ISO, 2014: **Error! Reference source not found.**). The scope of TC147 is standardization in the field of water quality, including definition of terms, sampling of waters, measurement and reporting of water characteristics. Excluded are the limits of acceptability for water quality.

Table 1-1: Summary of ISO TC147 for "Water Quality" activity

Total number of published ISO standards related to the TC and its SCs (number includes updates):	282
Number of published ISO standards under the direct responsibility of ISO/TC 147 (number includes updates):	2
Participating countries:	40
Observing countries:	50

Toxicity related standards are dealt with under ISO TC147/SC5 (Table 1-2) responsible for "Biological Test".

Table 1-2: Summary of ISO TC147/SC5for "Biological Methods" activity.

Number of published ISO standards under the direct responsibility of ISO/TC 147/SC 5 (number includes updates):	64
Participating countries:	30
Observing countries:	13

See Appendix B for a list of published aquatic toxicity standards and projects under the direct responsibility of the ISO/TC 147/SC 5 Secretariat (See ISO webpage http://www.iso.org/iso/home/store/catalogue_tc/catalogue_tc_browse.htm?commid=52972 for a list of published aquatic toxicity standards and projects under the direct responsibility of the ISO/TC 147/SC 5 Secretariat).

1.2.4 CONVENTIONS AND AGREEMENTS

1.2.4.1 Convention: Protection of the Marine Environment of the North-East Atlantic (OSPAR)

The Convention for the Protection of the Marine Environment of the North-East Atlantic (the "OSPAR Convention") was opened for signature at the Ministerial Meeting of the former Oslo and Paris Commissions in Paris on 22 September 1992. The Convention entered into force on 25 March 1998. It has been ratified by Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, Netherlands, Norway, Portugal, Sweden, Switzerland and the United Kingdom and approved by the European Union and Spain.

A submission titled “Background Document concerning the Elaboration of Programmes and Measures relating to Whole Effluent Assessment” featured at the OSPAR (2000) Commission Meeting. The following quote on Whole Effluent Assessment (WEA) is an indication of the approach to toxicity assessments in member countries:

“The Contracting Parties have developed different approaches to applying bioassays for wastewater evaluation. The emission-based approach requires that wastewater discharged into receiving water must be treated to meet certain defined limiting criteria based on Best Available Technology (BAT).

The water quality-based approach starts out from the actual or desirable state of the receiving water. Some of the national strategies applied combine both approaches in tiered assessment”

Among the Contracting Parties, it is widely accepted that a battery of toxicity tests covering the different trophic levels is needed for evaluations. This test battery should be defined according to the intended purpose (e.g. screening, characterisation and regulation of wastewater discharges). The report contains extensive data on toxicity test application by Contracting Parties. Recommended use is based on the known application of the tests and level of standardisation. In the section dealing with the status of WEA for the Contracting Parties, the status of WEA in the Contracting Parties' countries is summarised on the basis of the information given in the literature and the results of the survey performed by the German Federal Environmental Agency (OSPAR, 2000).

The following acute tests are most commonly used by the Contracting Parties: Fish (*Leuciscusidus, Brachydanio orerio, Cyprinus carpio, Dicenthrarcus palrax, Gasterosteus aculeatus, Salmo trutta* and *S. salar*), daphnids (*Daphnia magna, D. pulex*), algae (*Scenedesmus subspicatus, Raphidocelis subcapitata*) and bacteria (*Vibrio fischeri, Pseudomonas putida*, activated sludge, anaerobic digester sludge) (OSPAR, 2000).

1.2.4.2 Control of hazardous substances in the Baltic Sea region (COHIBA)

In all the participating countries of the COHIBA project, the EU legislation acts as a general framework for wastewater control. According to the Water Framework Directive (2000/60/EC) all water bodies should reach a good chemical and biological status by 2015 (COHIBA, 2010).

In the EU Water Framework Directive (2000/60/EC) (WFD), however, the evaluation of chemical status is based on single-substance approach and the evaluation of biological status is based on structure and species-richness of certain groups of water biota. No toxicity based criteria are included in the WFD. Pollution control requirements on WFD (article 16) have resulted in Decision 2455/2001/EC list of priority substances and Priority Substances Directive (2008/105/EC). These both stipulate on eliminating or reducing certain substances and are based on the single substance approach, but do not take into account the combined effects of chemicals.

EU Marine Strategy (Directive 2008/56/EC) provides a framework to reach good status of marine areas in the EU, but does not enact on reducing toxicity of effluents discharged into these marine areas. The directive on management of urban wastewater (91/271/EEC, see also 98/15/EEC) sets limits for nutrients and single hazardous substances and a few other parameters (such as oxygen demand), but does not regulate the eco-toxicity of whole effluents or combined effects of the chemicals under regulation (COHIBA, 2010).

Hazardous substances are one area of special concern in the Baltic Sea Action Plan (HELCOM, 2007). The action plan is mainly based on identification and reduction of single substances. In HELCOM recommendations concerning the wastewater discharges of chemical (recommendation 23/11) and textile industry (recommendation 23/12) and pesticide manufacturing (recommendation 23/10) there are also

toxicity limit value recommendations. In the recommendations 3-4 different toxicity test are listed (toxicity to *Daphnia magna*, algae, bacteria and fish) but the discharges need to select only 2 tests. All these recommendations consider only acute toxicity and the limits are far too lenient (COHIBA, 2010).

Nutrient content of effluent, concentrations of certain hazardous substances and a few other parameters, such as biological oxygen demand, are regulated. However, there are a few exceptions to this: the whole effluent toxicity of industrial discharges is regulated in some countries in the Baltic region. In all the Baltic countries municipal wastewaters are subjected to monitoring and control based on guidelines for chemical concentration data only (COHIBA, 2010).

1.2.5 RESEARCH MEETINGS AND SYMPOSIA

1.2.5.1 International Symposium on Toxicity Assessment (ISTA)

The biennial ISTA conferences provide a platform to present the latest data and discuss developments on all facets of environmental toxicology and eco-sustainability. ISTA brings together researchers, professionals, administrators, regulators, NGOs and policy-makers to exchange ideas, identify research and resource needs for the better management of ecosystem and public health, and to discuss strategies addressing consumer demands using environmentally friendly and sustainable industries. It also provides opportunities for networking and potential international collaborative research partnerships. Scientific themes in the current programs generally include the following:

- Biomarkers and bio-indicators
- Biotoxins
- Chemical toxicities and mechanisms of action
- Chemicals of global and emerging concerns
- Environmental chemistry and green chemistry
- Environmental safety and public health
- "Omics" and bioinformatics in (Eco)toxicology
- Regulatory toxicology
- Remediation and mitigation
- Risk assessment and environmental management

The close relationship between South African researchers and the conference resulted in the hosting of two of the sixteen conferences in South Africa. ISTA9 was hosted by the CSIR in Pretoria in 1999 and ISTA16 was hosted by the University of the Western Cape in Cape Town during 2012. ISTA9 was attended by 110 delegates from 31 countries. South Africa understandably had the largest contingent of delegates (34), followed by 10 delegates each from Canada and Japan. The others came from all corners of the globe. A total of 102 papers were presented, which included 62 platform and 40 poster papers.

1.2.5.2 Society of Environmental Toxicology and Chemistry (SETAC)

Interaction and communication between decision makers and researchers is of major importance in the development and implementation of measures to establish and maintain viable and quality water resources. SETAC was identified as one of the reputable and experienced societies which can play a prominent role to implement bio-assays in South Africa. The brief introduction below illustrates the value of the Society. In the 1970s there was no forum for interdisciplinary communication among environmental scientists such as biologists, chemists, toxicologists as well as managers and engineers others interested in environmental issues. SETAC was founded in North America in 1979 to fill this gap. Based on the dynamic growth in the

Society's membership, meeting attendance and publications, the forum was clearly needed. SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1990, and in Brussels, Belgium, established in 2003.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The society by-laws mandate equal representation from these three sectors for officers, World Council, Geographic Unit Boards of Directors and Councils, and Committee members and governance of activities. The proportion of members from each of the three sectors has remained nearly equal over the years. Membership has increased from 230 Charter Members in October 1980 in North America to the present level of nearly 5,500 members from more than 100 countries. Participants and technical presentations at SETAC annual meetings have increased from 470 attendees and 86 presentations in 1980 to some 2,500 participants and nearly 1,900 presentations at annual meetings in North America and Europe, with smaller, but still substantial participation at biennial meetings in Asia/Pacific, Latin America and Africa.

SETAC publishes the following two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations:

- *Environmental Toxicology and Chemistry*, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1980 to a monthly publication of nearly 3,000 pages annually.
- *Integrated Environmental Assessment and Management*, launched in 2005 to bridge the gap between scientific research and it application in environmental decision-making, regulation and management, has become a well-respected quarterly publication of 700 pages annually.

Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies. With the establishment of geographic units in North America, Europe, Asia/Pacific, and Latin America by 1999, the Society moved to create a 15-person SETAC World Council (SWC) in 2002. The geographic units are represented on the Council, with representation keyed to their relative shares of membership. Africa joined the global organization as a full-fledged geographic unit in May 2012.The decision came after a decade of SETAC activity in Africa, including the formation of a SETAC Africa Branch within SETAC Europe and a series of biannual meetings, most recently in Buea, Cameroon in May 2011, and prior to that in Kampala, Uganda (2009), Arusha, Tanzania (2007) and in South Africa. The primary goals of SETAC Africa are:

- To promote research, education, training and development of the environmental sciences, specifically environmental toxicology and chemistry, hazard assessment and risk analysis.
- To encourage interactions among environmental scientists and disseminate information on environmental toxicology and chemistry and its application to the disciplines of hazard and risk assessment.
- To sponsor scientific and educational programs and provide a forum for communication among professionals in government, business, academia and other segments of the environmental science community involved in the use, protection, and management of the environment, and the protection and welfare of the general public.
- To promote the development and adjustment of principles and practices for sustainable environments, considering appropriate ecological, economic and social aspects adapted to African problems and conditions.

Currently SETAC Africa's membership approaches 100 with the majority of the members coming from South Africa.

1.2.5.3 Microbiotest Symposium for Routine Toxicity Screening and Biomonitoring

The Proceedings of the "International Symposium on New Microbiotests for Routine Toxicity Screening and Biomonitoring" edited by (Persoone et al., 1999) represents a summary of pre 2000 advances in techniques, new tests and application in many countries. The scientific papers, together with more than 100 oral and poster contributions by eminent internationally recognized eco-toxicologists underlined the importance and global application of bio-assays in water management. Curbing costs of eco-toxicological tests especially those used for routine control and monitoring purposes and the availability of acceptable stocks of live test organisms featured prominently in many of the discussions. Technology transfer and a clear understanding of an effect based approach were deemed of the utmost importance. Basic considerations in the development and selection of toxicity tests are that the purpose of the assay should be kept in mind. Tests for screening, regulatory requirements and predictive hazard assessment each have a different set of requirements as to test precision, test organism choice, exposure time and cost (Persoone et al., 1993).

1.2.5.4 Report: International Comparative Study on Toxicity Assessment of Chemicals

A research report edited by Isao Aoyama (Research Institute for Bio-resources, Okayama University) comprises of a host of scientific papers on toxicity issues. The unification of eco-toxicological research in Japan by the establishment of the Japanese Society of Environmental Toxicology in 1997 was considered "most desirable" (Aoyama, 2000).

In 2000, surveys conducted in 38 countries revealed that the majority have no regulations pertaining to the use of bioassays to monitor the discharge of toxicants in receiving water. In many countries effluent discharges are "tokenly" and unofficially monitored (Dutka, 2000). Several of the contributions indicated that research on the methodology of toxicity testing and the application thereof was receiving more attention in most of the countries and states surveyed and provided details on the *status quo* in Canada and South Africa. (Slabbert, 2000). Cost, infrastructural deficiencies and lack exposure to the real value of bioassays were quoted as factors which inhibited development in this field.

1.2.6 INTERNATIONAL BIOASSAY APPLICATION

1.2.6.1 Australia and New Zealand

The Australian National Water Quality Management Strategy (NWQMS) aims to achieve the sustainable use of Australia's and New Zealand's water resources by protecting and enhancing their quality while maintaining economic and social development. The NWQMS is a joint strategy developed by two Ministerial Councils: the Agriculture and Resources Management Council of Australia and New Zealand (ARMCANZ) and the Australian and New Zealand Environment and Conservation Council (ANZECC). The Australian Water Quality Guidelines for Fresh and Marine Waters (ANZECC, 1992) was released in 1992 as one of the first guideline documents. Since the ANZECC Guidelines were published in 1992 there have been a number of important advances including (ANZECC, 2000):

- Major policy initiatives at federal and state level that, combined with the National Water Quality Management Strategy, have increased the focus of attention on ecologically sustainable management of water resources in Australia and New Zealand.
- A trend towards a more holistic approach to the management of aquatic systems.
- An increased use of biological indicators to assess and monitor the "health" of aquatic ecosystems.

Australia and New Zealand both have a regional or local government framework in place. The political boundaries imposed within Australia place most of the responsibility for the management of natural

resources with the states and territories. In New Zealand primary responsibility for water management rests with regional councils.

Water resource management is best implemented by integrating national, state and regional powers and responsibilities, and by using complementary water quality planning and policy tools. After all available and technical information has been collated for a defined water body; the steps listed below are followed to implement a broad national management strategy at a local level (this may be relevant in the current South African situation).

- Identify the environmental values that are to be protected in particular a water body and the spatial designation of the environmental values (i.e. decide what values will apply where).
- Identify management goals and then select the relevant water quality guidelines for measuring performance. Based on these guidelines, set water quality objectives that must be met to maintain the environmental values.
- Develop statistical performance criteria to evaluate the results of the monitoring programs (e.g. statistical decision criteria for determining whether the water quality objectives have been exceeded or not).
- Develop tactical monitoring programs focusing on the water quality objectives.
- Initiate appropriate management responses to attain (or maintain if already achieved) the water quality objectives.

The elements of this management strategy can be incorporated into comprehensive planning practices such as integrated (or total) catchment management plans (ICM or TCM) or can remain relatively small-scale plans for local areas. However, there must be consultation with stakeholders and the effective use and integration of a multi-disciplinary array of skills and knowledge to achieve success. With respect to the fifth point above, the management responses will depend on the issue of concern, the cause(s) of the poor water quality and the available tools, and should be negotiated and agreed upon by the local or regional stakeholders.

In Australia, strategic management can be in the form of catchment management plans or state or national policies (e.g. statutory Environmental Protection Policies) and in New Zealand, Regional Policy Statements, regional plans or National Policy Statements, based on the agreed environmental values and their associated water quality objectives. Regulation could be achieved through discharge consents and codes of practice designed to ensure water quality objectives are not exceeded and taking into account cumulative impacts from all sources. The monitoring programs identified in the fourth point above should be maintained during and after implementation of the agreed management response(s), to evaluate their performance in achieving the water quality objectives and hence the management goals. This process should be iterative and on-going to ensure the environmental values continue to be sustained.

The NWQMS outlines a three-tiered approach to water quality management at:

- the national level — a vision of achieving sustainable use of water resources by protecting and enhancing their quality while maintaining economic and social development together with overarching national guidelines for minimum water quality;
- state or territory level — implementation through state water quality planning and environmental policy processes, to provide a planning and management framework with goals and objectives consistent with the agreed national guidelines;
- regional or catchment level — complementary planning, with local or catchment management strategies developed and implemented by the relevant stakeholders. Regional communities are encouraged to participate in identifying the local environmental values and to monitor and report on progress and performance of the plans.

To underpin water resource management at the national, state and territory levels in Australia, a range of legislative and regulatory tools are being used. Examples include state and territory water and land resources management Acts, environment protection Acts, the development of water quality guidelines focused on state and territory water resources and the development of national environmental protection measures. Each state or territory uses its own water quality planning and environmental policy tools to establish a framework compatible and consistent with the agreed national guidelines.

In New Zealand, these guidelines are designed to assist water managers with the implementation of the Resource Management Act (ANZECC, 2000) which gives regional councils primary responsibility for water management. The RMA empowers councils to develop statutory plans and local laws for water management. The RMA also enables central government to develop national policy and standards on a statutory basis. Overall the responsibility for water resource management rests with the community. The tools, strategies and policies developed to manage and protect environmental values should be applied in this wider context. In effect, there must ultimately be education and change in community's behaviour toward a more environmentally sustainable approach.

The responsibilities for monitoring water resource quality should not always rest with government alone and ideally would be shared with the dischargers/users of the environment in question (these shared responsibilities could extend to the waters beyond the mixing zone of outfalls). Many community and catchment groups have already become involved in or taken responsibility for, water quality monitoring programs and are developing management strategies to maintain or improve their water resources.

The current NWQMS approach recommends moving away from relying solely on chemical guideline values for managing water quality, to the use of integrated approaches and levels of protection (Table 1-3), comprising:

- chemical-specific guidelines coupled with water quality monitoring;
- direct toxicity assessment; and
- biological monitoring.

Direct toxicity assessment is a useful tool that can be used in these circumstances, although it is mainly used to assess the toxicity of complex effluents and to derive guidelines for the amount of dilution required to safely discharge an effluent to aquatic environments. It can also be used as a monitoring tool for testing the ambient waters after they have received effluent discharges. The main advantage with using direct toxicity assessment is that it is not necessary to know the exact chemical make-up of the test effluent, and the interactions between the components, to determine potential impacts.

Table 1-3: General framework for applying levels of protection for toxicant to different ecosystem conditions (ANZECC, 2000).

Ecosystem condition	*Level of protection*
1. High conservation / ecological value	• For anthropogenic toxicants, detection at any concentration could be grounds for source investigation and management intervention; for natural toxicants background concentrations should not be exceeded.[a] Where local biological or chemical data have not yet been gathered, apply the 99% protection levels as default levels. Any relaxation of these objectives should only occur when comprehensive biological effects and monitoring data clearly show that biodiversity would not be altered. • In the case of effluent discharges, Direct Toxicity Assessment (DTA) should also be required on the effluent. • Precautionary approach taken to assessment of post-baseline data through trend analysis or feedback triggers.
2. Slightly to moderately disturbed ecosystems	• Always preferable to use local biological data (including DTA) to derive guidelines. If local biological effects data unavailable, apply 95% protection levels as default, low-risk trigger values.[b] 99% values are recommended for certain chemicals.[c] • Precautionary approach may be required for assessment of post-baseline data through trend analysis or feedback triggers. • In the case of effluent discharges DTA may be required.
3. Highly disturbed ecosystems	• Apply the same guidelines as for slightly-moderately disturbed systems. However, the lower protection levels provided in the Guidelines may be accepted by stakeholders. • DTA could be used as an alternative approach for deriving site-specific guidelines.

[a] This means that indicator values at background and test sites should be statistically indistinguishable. It is acknowledged that it may not be strictly possible to meet this criterion in every situation.
[b] For slightly disturbed ecosystems where the management goal is no change in biodiversity, users may prefer to apply a higher protection level.
[c] 99% values recommended for chemicals that bioaccumulate or for which 95% provides inadequate protection for key test species. Jurisdictions may choose 99% values for some ecosystems that are more towards their slightly disturbed end of the continuum.

1.2.6.2 Austria

In Austria aquatic toxicity testing is imposed to estimate the potential hazards of the discharge of municipal and industrial effluents into the receiving waters. The discharges of the treatment plants as well as the receiving waters are monitored periodically and regulated continually by the Austrian authority. Standard toxicity methods are carried out according to the Austrian and German guidelines (ÖNORM, DIN). These include aquatic toxicity tests, which are acute (short) and chronic (sub-lethal) tests. The following toxicity tests are used for the investigation of the water and wastewater:

- Acute tests: Luminescence inhibition test with bacteria (DIN 38412-L34, March 1991, DIN 38412-L341, Oct. 1993, ÖNORM M 6609, June 1993), Immobilization test with Daphnia (ÖNORM EN 26341, ÖNORM M 6264, Jan. 1984) and the Fish lethality test (ÖNORM M 6263 part 1 or 2 Nov. 1987).
- Chronic test: Algal growth inhibition test (DIN 38412-L33, March 1991).

The frequency of the investigations required in the Austrian legislation is once in five years, unless the individual regulations are more strict (personal communication, Persoone).

The G-values are used as safe limits for toxicity tests of the effluents and receiving waters. The G-values are dependent on the type of the discharges (effluents). They are GL (bacteria), GD (Daphnia), GF (fish) and GA (algae). Most of the G-values of the industrial effluents such as effluents of pulp and paper, textiles, leather, organic and inorganic chemicals and detergents industries have GF = 2, GL and GD = 4, GA = 8, while the effluents of the pharmaceutical and pesticide manufacturing industry have to observe the legal requirements of GA16. In the case of the municipal effluents, only fish test is required and the GF should be less than 2 (ÖNORM, DIN).

1.2.6.3 Belgium

Wastewater regulatory practice in Belgium is organised in three regions: Flemish, Brussels and Walloon (OSPAR, 2000). The Industrial wastewater discharge in the Flemish and Walloon region is subject to three levels of mandatory conditions (Vlarem, 1995; Goenen, 1996). There are:

- General conditions for discharge of wastewater into surface waters and into sewers (limits on pH, BOD, temperature, suspended solids, extractable substances, dangerous substance according to EU directive 76/464/EEC).
- Sectoral conditions based on chemical analysis have been adopted in the Flemish Region for several wastewater sectors to describe Best Available Techniques (BAT).
- Particular conditions are more stringent than the former two and are aimed to protect the particular ecological equilibrium of the receiving water.

The Brussels and the Walloon regions apply the federal legislation to establish permits for wastewater discharges for different sectors including chemical, pharmaceutical and as well as petrochemical industry.

In some studies on Whole Effluent Toxicity (WET) testing alternative tests (Tox kits, microliter plate test with algae) were used for which standard procedures from the developers exist. These were compared with OECD or ISO standardised acute toxicity tests *with Vibrio fischeri, Daphnia magna* and *Oncorhynchus mykiss.* Some non-acutely toxic effluents were evaluated for chronic toxicity (*Daphnia* reproduction and zebrafish short-term test), genotoxicity and persistence. Studies for endocrine disrupting effects with a recombinant yeast estrogen assay (Tanghe et al.,1999) and comparative work with this same yeast estrogen assay and the human recombinant breast cancer cell line (MVLN) (Witters *et al*., 1999) with environmental samples have been performed, but up to now no results with effluents from industrial sectors are reported (OSPAR, 2000).

1.2.6.4 Botswana

In line with other members of the Southern African Development Community (SADC), Botswana devised a National Water Master Plan (NWMP) and undertook a series of institutional and legal reforms throughout the 1990s so as to make water resources use more equitable, efficient and sustainable. The stated goal is to work toward Integrated Water Resources Management (IWRM) in both policy and practice. Currently bioassays do not feature in policy guidelines or legislation

1.2.6.5 Brazil

According to Professor Gisela Umbuzeiro and Fernando Mello (personal communication) eco-toxicological standards have been applied by some Brazilian environmental state agencies since 1990. Eco-toxicological parameters were included and published in federal regulations in 2005. These parameters were upgraded in 2011.

A case by case risk based approach based on US EPA is applied, with sampling and measurements where the effluent is discharged. The objective is to set up a standard to protect the quality of the receiving

environment, also considering its dilution capacity. Federal laws determine that each state environmental agency has the freedom to define its own specific criteria, prior to which the federal model should be used.

At present some states use the federal criteria (risk-approach) and other prefer to use the hazard approach (German model). This is based on load, reducing emissions using the best available and economically feasible technology. We concluded that both approaches should be integrated.

In general, the eco-toxicological assays must include species of two different trophic levels. Federal prescriptions state that the effluent concentration in receiving waters (ECRW) must be equal or lower than de NOEC or EC50/10 (that is for receiving waters where the protection of aquatic life is an objective). The Sao Paulo state is more strict and use ECRW < NOEC/10 or EC50/100. In addition it is possible to integrate this with the hazard approach, setting standards for each industry group, based on the best available technology.

As for applications, sampling procedures and sampling points, each environmental agency sets their own rules. The sampling procedures are often extracted from international sample protocols and according Brazilian legislation the sampling must be done in the final effluent.

Each environmental agency sets its own monitoring frequency. It is recommended that the effluent variability be considered when determining the frequency and type of sample (simple or composite sample). Laboratories that conduct the assays must be certified in good laboratorial practices (SANS ISO/IEC, 2005) or are in the process of certification). General guidance on the requirements of a laboratory and QA/QC can be found in Appendix A.

The federal regulation requires acute and chronic effects to be determined, but does not prescribe the organisms to be used. The rule is to use the result of the most sensitive among the tested organisms. The state regulation of Rio Grande do Sul prescribes that mutagenic endpoints and set progressives goals be reached in relation to time.

1.2.6.6 Canada

The use of biological toxicity procedures has been developed based on data acquisition for acute toxicities, towards physicochemical and biological parameters which are regulated and monitored by industrial sectors in the 1970's, and finally according to compliance monitoring and hazard assessments that were conducted during the 1980's (Blaise et al*.,* 1988). The Environmental Protection Service administers several statutes (Fisheries Act; International Boundary Waters Treaty Act, Environmental Contaminants Act) (Sergy, 1987). Canada supports biological testing within eco-toxicological approaches, in order to characterise effluents and controls, and has reviewed its national bio-testing capabilities to implement uniform assessments on a national scale (Blaise et al,1988). The Ontario Ministry of the Environment re-assessed strategies for effluent control are applied by way of the Municipal and Industrial Strategy for Abatement (MISA) programme. Although the emphasis is on toxicants, MISA controls conventional pollutants as well (Sergy, 1987). Critical assessments were made on the use of biological tests in the MISA programme. Chapman et al. (2011a) noted that the removal of acute lethal toxicity *via* Best Available Technology Economically Achievable (BATEA) is considered to be the first step. The 96 hour rainbow trout and the *Daphnia* lethality tests were the most likely regulatory tests. Other bio-monitoring tools which measure chronic, genotoxic and sub-lethal effects were also under evaluation at MISA pilot site studies (Chapman et al., 2011a). These then play a role in the defining water quality based controls. The MISA Working Group stated: "As a minimum, industrial and municipal discharges must be non-acutely lethal to fish. Since the impact of toxic discharges on aquatic organisms ranges from acute lethality through sub-lethal toxicity leading to adverse chronic effects, further appropriate effluent bio-monitoring tests should be applied (and/or developed)on an industry or sector-specific basis" (Sergy, 1987). In 1987 the Canadian Environmental Protection Act received first reading in

the House of Commons. Both the spirit and letter of the Act demanded the use of toxicity tests and bio-monitoring procedures (Sergy, 1987).

The Canadian Environmental Protection Act, (Scroggins, 1999) was made available in March 2000 and its objective was to prevent pollution and protect the environment and human health. The act requires that toxicity testing for the registration of new substances in order to conform to the Organisation for Economic Cooperation and Development (OECD) standards of Good Laboratory Practice (GLP). 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 has two main objectives:

- The virtual elimination from the environment of toxicants that are persistent and bio-accumulative, and
- the management of other toxic substances to prevent or minimise their release into the environment (Chapman et al., 2011a).

For toxicity tests to meet the regulatory requirements, the Canadian Inter-Governmental Aquatic Toxicity Group proposed the development and standardisation of a set of single species aquatic toxicity tests (Chapman et al., 2011a). 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 (EC, 2000a).
- Biological Test Method: Reference Method for Determining Acute Lethality of Effluents to *Daphnia* (EC, 2000b).
- Biological Test Method: Reference Method for Determining Acute Lethality of Sediment to Marine or Estuarine Amphipods (EC, 1998).
- Biological Test Method: Reference Method for Determining the Toxicity of Sediment Using Luminescent Bacteria in a Solid-Phase Test (EC, 2002).

Environment Canada has defined 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 methods are frequently restricted to testing requirements associated with specific regulations (Chapman et al., 2011a). 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.

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

The Ontario Safe Drinking Water Act can serve as an example to illustrate the nature and scope of detailed prescriptions and policy where water resource control and monitoring is administered by executive authorities and agencies. Of particular interest inter- agency co-operation whereby officers acting under the auspices of the EPA, Nutrient Management Act, Ontario Water Resources Act, Pesticides Act and Toxics Resources Act are authorised to undertake inspections relating to the discharge of toxicants.

A wide range of biological tests are conducted in the Canadian Federal Environmental Protection Laboratories. Generally, the tests employed measure both acute and chronic toxicity with their corresponding lethal and sub-lethal effects and include several representative species of different trophic levels (Mac Gregor and Wells, 1984; Sergy, 1987). The amount, manner and effectiveness of use of the tests are not the

same in the different laboratories. This is because of different federal regional strategies, mandates, expertise and budgets (Blaise et al., 1988). The types of tests are as consistent as possible with OECD guidelines, US EPA and ASTM methods. The application of the biological tests in environmental protection activities includes four major steps: problem identification; problem assessment; control or intervention; and control evaluation (Sergy, 1987). The application of bioassays for drinking water protection in Canada is limited to research institutions like the National Water Research Institute, Canada Centre for Inland Waters and Burlington. The battery approach is followed using a range of tests, including several microbial tests (Dutka and Kwan, 1981; OSPAR, 2000).

Canada ranks effluents according to their environmental hazard potential and thus uses a water quality-based approach (Tonkes et al., 1995). ”Environment Canada” developed an evaluation system, based on effluent toxicity testing capable of ranking the environmental hazards of industrial effluents. No allowance has been made for in stream dilution; therefore no risk assessment of environmental effects is modelled. The ecotoxicological test systems used are: *Vibrio fischeri*, *Selenastrum capricornutum*, and *Ceriodaphnia dubia*. Additionally, genotoxicity tests (SOS-chromo-test) are performed. All results are expressed as threshold values (LOECs) and subsequently transformed to toxic units. All test systems are considered to be necessary to describe potential risks of effluents. Industrial sectors covered under national effluent regulation include pulp and paper, metal mining and petroleum refining. Toxicological testing is required under regulations for each of these sectors as either a compliance requirement (i.e. rainbow trout acute lethality) or as a legal monitoring requirement (i.e. battery of sub-lethal toxicity tests). At the provincial level, many industrial and municipal facilities are required to conduct aquatic toxicity testing as a condition of their effluent discharge permit.

1.2.6.7 Denmark

Toxicity testing was implemented into practice in county councils as an unofficial guideline in the 1990’s. At present discharge of industrial wastewater is regulated according to the consolidated Environmental Protection Act (625/1997) (OSPAR, 2000).

About 100 industrial companies discharge wastewater directly into surface waters. Direct discharge permits for wastewater are issued by the county councils. Discharge into municipal sewers has to be licensed by the local municipalities. In cases, Best Available Technology (BAT) as well as potential ecological risks is stated to be regarded.

There are guidelines for whole effluent toxicity assessment and regulation of industrial wastewaters (OSPAR, 2000; Pedersen et al., 1994). Although these guidelines are not statutory, they are commonly applied to larger industries. Different criteria have been applied to different industries and how they should meet toxicity requirements, based on dilutions, flow and other factors. Pedersen et al. (1994) describe the range of eco-toxicity tests employed in Denmark, which include algae, marine copepods (2 spp.) (lethality, reproduction and life cycle), *Daphnia magna* (acute lethality and life cycle) and fish (acute lethality and early life stage). The discharger has the responsibility to prove compliance (Pedersen et al., 1994). In Denmark the emphasis is on water quality, i.e. the site specific approach. Both acute and chronic toxicity is taken in account, but the guidelines do not include endocrine disruption or genotoxicity.

Since the 1980s an eco-toxicological characterisation survey of 23 major industrial wastewater discharges has been performed covering the major Danish industrial enterprises (Pedersen et al., 1994). Usually 3-5 species were used, representing algae, crustacean and fish species. In some studies toxicity on bivalves (Blue mussel), bacteria (*Vibrio fischeri*, inhibition of respiration/nitrification of activated sludge) and plants (Cress, *Allium*) was also evaluated.

Acute test data on three species allows calculation of an acute *no-effect concentration* (NOEC a) which will not give unacceptable acute-toxic effects. Chronic data allow calculation of a chronic NOEC (NOEC c), applicable to the maximum effect concentration over 4 days. Using data from 5 species allows full quantification of effects but more complex tests may be applicable if the acute and chronic test stages reveal uncertainties.

In the 1990's a detailed strategy for effluent toxicity testing was developed for freshwater and the marine environment (Pedersen et al.,1994) and is currently used as an unofficial guideline. The risks for the receiving aquatic environment are assessed by comparing the Predicted Environmental Concentrations (PEC) with the Predicted No-Effect-Concentration (PNEC) of the effluent. The principles for investigating industrial wastewater and performing environmental risk assessments are based on three levels (Pedersen et al., 1999):

- Evaluation based on existing knowledge (inventory of the chemicals, mass balances, emissions);
- Standardised investigations of the wastewater (acute toxicity tests with 3 different species, aerobic stabilisation, HPLC screening for bio-accumulative substances, evaluation of initial dilution);
- Specialised investigations, of the wastewater or prioritised substances contained therein (chronic toxicity, toxicity to organisms from specific compartments, biodegradation, bio-accumulation tests with fish).

At each investigation step an environmental risk assessment is performed focussing on single substances but also considering the complex wastewater itself. Assuming the same principles as for assessing the risks of chemical substances based on EU technical guidance documents, a PEC/PNEC approach is performed. (OSPAR, 2000).

1.2.6.8 Finland

Whole Effluent Toxicity (WET) testing in Finland focuses on pulp and paper mill effluents. A wider range of biotests (freshwater fish acute and chronic toxicity, *Daphnia magna* chronic toxicity) is used in research and development projects aimed at determining the wastewater situation in various industrial sectors (OSPAR, 2000).

Whole Effluent Assessment has been applied in few environmental permits of industrial effluents, but this is not a common practice and there are no statutory toxicity limits or toxicity reduction measures. Standardised internationally accepted bioassays (such as ISO methods) with *Daphnia magna*, *Vibrio fischeri, Pseudomonas putida* and algae are used occasionally in compliance monitoring. Results are presented as EC50 values. The monitoring takes place at partial streams and the outlets after mixing with cooling water.

1.2.6.9 France

In France industrial effluents are regularly monitored for acute toxicity with daphnids. The toxicity data are used as a base for discharge taxation (De Zwart, 1995). Both the dilution capacity of the receiving water and the potential use of the water are taken into account. This means, a combination of the emission-based and the water-quality approach is applied. Discharge permits also depend on EU Directives. For more polluting industries national limit values based on BAT and BATNEEC (Best Available Techniques not Entailing Excessive Costs) have been issued, which are considered as minimum values. Group parameters (AOX, metal, BOD) are also included (Tonkes et al., 1995). A wide spectrum of nationally (AFNOR) or internationally (OECD, ISO) standardised acute and chronic biotests including bacteria, algae, *Lemna*, rotifers, various daphnid species, freshwater as well as marine fish species is used to determine LC_{50} and EC_{50} values at the outlets after mixing with cooling or other water and in the receiving waters close to the outlets. Most of these are employed only occasionally in discharge permits procedures, in water quality monitoring or in the framework of research and development programs.

Acute toxicity for *Daphnia magna* and inhibition of bacterial luminescence of *Vibrio fischeri* are also determined at the outlets of treatment facilities prior to mixing. There are only a few reports on WET testing available focussing on *Daphnia magna* and *Vibrio fischeri*. Along with the acute toxicity test with daphnids it is proposed to add the photo bacterium assay, chronic toxicity and a test on muta-genicity to the set of required bio-criteria in regulatory practice (De Zwart, 1995). Furthermore a test scheme based on a series of eco-toxicity tests and analytical identification of organic pollutants of concern is under development by industrial researchers.

Two samples are usually assessed: the existing whole effluent for characterisation of the current impact of the treatment plant, and a modelled future effluent, based on available information on the new process or on pilot studies. Results of these risk assessments are used by the companies to evaluate the contribution of the new process to the impact of the plant on the receiving system and, if necessary, to make any improvements (Boutonnet et al., 1999; OSPAR, 2000).

1.2.6.10 Germany

In Germany, whole effluent assessment and whole effluent toxicity limits have been part of wastewater control since 1970's. WEA (Integrating Controlling of Effluents, ICE) has been routine practice since 1976. The environmental policy emphasises the emission-based approach. A water quality-based approach has been developed in parallel.

Two important federal laws constitute essential elements of water pollution control:

- the Federal Water Act (WHG) of 1957 and
- the Wastewater Charges Act (AbwAG) of 1976 (OSPAR 2000)

The range of tools available under these acts has been steadily expanded and improved. They have reached a high standard despite the fact that federal legislative powers are restricted to the enactment of framework provisions (Article 75 German Basic Law). Water quality is controlled by the Federal Water Act of 1957, as amended in 1976.

States have set up water authorities who are responsible for river water quality. Local authorities are responsible for discharges into sewers. The General Administrative Directive for effluents includes guidelines on fish toxicity for some effluents, expressed in terms of a dilution factor (for which all the fish survive under the conditions specified in the standard method). Recent information) indicates that water authorities will in future rely more extensively on a range of biological tests, which will move Germany away from its current strict reliance on emission parameters, towards a Quality Standards for Receiving Waters Approach. It is envisaged that fish, *Daphnia,* algal and luminescent bacterial tests will be used in a few years' time to monitor all waters (Slabbert et al, 1998a & b).

According to the German Federal Water Act, discharge permits shall be granted only if the waste load is kept at least on the current BAT level. The requirements based on BAT are established by the federal government in the appendices of the Wastewater Ordinance (AbwV) for the different industrial branches and processes and updated according to further development of BAT. There are two legal regulations where WEA is applied in wastewater evaluation:

- The AbwV (Ordinance on Requirements for the Discharge of Wastewater into Waters, Wastewater Ordinance – AbwV) based on WHG. Within the AbwV, 10 freshwater biotests are included for which wastewater adapted national standards or ENISO standards exist. Included are 5 tests that deal with bio-degradation (e.g. BOD, modified Zahn Wellens tests with 3 to 28 day test duration, treatment

plant simulation model) as well as short term toxicity tests with *Leuciscusidus*, *Daphnia magna*, *Scenedes mussubspicatus* and *Vibrio fischeri*, representing different trophic levels in the aquatic environment. Since 1999 the UMU genotoxicity test has also been included.

- The Wastewater Charges Act (Act pertaining to Charges levied for Discharging Wastewater into Waters). In the AbwAG an acute fish toxicity test is implemented for industrial and municipal direct discharges to a receiving water body. Charges are based on COD, heavy metals, nitrogen, phosphorus, AOX and fish toxicity. Specific charges are calculated from pollution units. For example one pollution unit (about 40 Euro) corresponds to a load of 20 g mercury or 500 m^3 wastewater with acute fish toxicity with a LID (Lowest Ineffective Dilution) of 6. For a limit LID of 2 no charge based on fish toxicity is imposed.

The following bioassays (Table 1-4) are provided for in the Wastewater Ordinance:

Table 1-4: Regulatory practice including biotests in Germany (OSPAR, 2000).

Annex	*Wastewater source sector*	Leuciscus idus *[LID]*	Daphnia magna *[LID]*	Scenedesmus subspicatus *[LID]*	Vibrio fischeri *[LID]*	*UmuC Genotoxicity [LID]*	*Elimination*
22	Chemical and pharmaceutical	2 (DC)	8 (DC)	16 (DC)	32 (DC)	1.5 (DC)	90-95% TOC (DC, ID)
25	Leather and fur	2-4 (DC)					90% COD 98% BOD
30	Manufacture of sodium carbonate	32 (ID)					
31	Cooling water				12 (DC)		
40	Metals	2-6 (DC)					
51	Landfill leachate	2 (DC, ID after treatment)	4 (ID after treatment)		4 (ID after treatment)		75% DOC (ID)
57	Raw wool washing	2 (DC)	2 (DC)				

LID: Lowest ineffective dilution
DC: Direct discharge to a receiving water
ID: Indirect discharge via public sewers to a wastewater treatment plant

Pursuant to German Wastewater Ordinance (Federal Ministry for the Environment, 2004) statutory toxicity limits concern various industry sectors such as chemical, metal, pulp and paper production as well as storage and management of solid waste. The limits are based on sector-specific BAT. The criteria in Wastewater Ordinance are only minimum requirements and the Länder (federal states) can set more stringent limits. Toxicity to fish eggs is the most commonly used toxicity criteria, but toxicity to *Daphnia* or algae and UMU-test are also applied. The implementation of WEA in Germany is of the source control type i.e. hazard-based approach (OSPAR, 2000). In the Wastewater Ordinance it is also clearly stated that compliance shall not be met by diluting wastewater before discharge. Compliance monitoring can be performed by the officials or as self-monitoring provided that the discharger uses officially recognised techniques (Federal Ministry for the Environment, 2004).

Discharge limits to different wastewater sectors are set in about 50 annexes of the Wastewater Ordinance. Depending on the emission spectrum, chemical analysis of 12 anions/elements, 24 cations/elements, 38 individual substances, and group parameters including AOX, TOC, COD as well as total nitrogen are measured. In about 30 wastewater sectors the fish toxicity test is part of the licensing of wastewater permits. BOD measurements are required in most of the wastewater sectors.

Currently the most developed concepts including biotests in discharge limits are those covering the chemical industry and the landfill leachates. For the latter the limits for aquatic toxicity for indirect discharges may be reached after treatment in a laboratory activated sludge treatment plant considering that degradable toxicities (e.g. due to high ammonium concentrations) do not affect surface water. In regulatory practice the Zahn-Wellens-Test is also used as a model for elimination processes in wastewater treatment plants. Additionally other bioassays can be demanded by local authorities within the discharge permit procedure in a case by case consideration. Unlike the testing of substances, the German approach for wastewater regulation is not based on risk assessments for the receiving waters.

Recently WET results of more than 10 000 samples from various industrial sectors were documented in detail in the proceedings of the OSPAR workshop held in Berlin in 1997 (Hagendorf and Brackemann, 1997, Diehl and Hagendorf, 1998). Extensive research projects for the evaluation of wastewater qualities in the textile, chemical, and pharmaceutical industries as well as in hospital wastewaters have been performed. In these activities, biotests were applied to assess possible risks regarding ecotoxicity, degradability and genotoxicity of the wastewater. Only a small number of test results have been generated in German with the UMU-test, since this test has only recently been implemented and employed. The current focus is on wastewater from hospitals, chemical industry and municipal wastewater plants.

Fundamental to the implementation of biotests in WEA in Germany is the precautionary principle (to do all that can be reasonably expected to prevent unnecessary risks) and the “Polluter Pays Principle (PPP -the principle that transfers the financial burden for the prevention and control of pollution on the party responsible for its generation). The emphasis of the German approach is on emission reduction at the source and does not include environmental risk assessment which takes into account the flow capacity of the receiving body.

Mixing or dilution may not achieve the limit values set in wastewater discharge permits for the different parameters. German experience over the last 23 years shows that this approach promotes the further development of BAT and has supported its use considerably. Coupling WET with the BAT guarantees equal treatment of discharges in the different branches of industry regardless of the water quality of the receiving waters. The guiding concept in emission control is the combined use of chemical group parameters, measurement of single substances and biotests. Requirements for the discharge of wastewater into waters are laid down in the appendices of Wastewater Ordinance.

The revenue accruing from wastewater charges may only be used for specific purposes connected with measures for maintaining or improving water quality. The *Länder* (provincial state governments) may stipulate that the administrative expenditure associated with the enforcement of the Wastewater Charge Act and of the *Länder´s*own supplementary provisions shall be paid for out of the revenue accruing from wastewater charges. In emission control the first aim is to avoid the presence of hazardous substances and undesirable effects in wastewater.

Standardised biotests developed and used for that purpose must be capable of detecting effects clearly, rapidly and cost-effectively. The results from these biotests are not expected to provide final evidence of an effect at ecosystem level and consequently they are not used for risk assessment procedures. The evaluation of toxicity tests follows the concept of Lowest Ineffective Dilution (LID) according to the informative annex of EN ISO 5667-16, which is exclusively applied in Germany. LID is the most concentrated effluent dilution at which there is no observed effect on the test organism, or there are only effects that do not exceed the test-specific variability. LID is expressed as the reciprocal value of the volume fraction of wastewater in the test dilution.

Currently biotests for other endpoints such as bioaccumulation, endocrine disruptors, immune toxicity, and mutagenicity (with eukaryotic cells) are in the development stage. In special cases, ambient toxicity close to the effluent discharge location is also determine, but not on a routine basis. In large rivers (such as the Rhine

and the Elbe) continuous biological monitoring devices (daphnids, dreissena) are in operation as early-warning systems.

1.2.6.11 Greece

According to Dr Vasilios Tsiridis (personal communication, 20014), there is no national legislation on whole effluent toxicity tests in water use licenses in Greece. Legislation for water has been harmonized with the European Water Framework Directive 2000/60/EC of October, 2000.

This Directive sets environmental quality standards suggesting safety factors based on acute and chronic ecotoxicity tests with fish, algae, daphnia or a representative organism for saline waters. With the exception of wastewater there is no national legislation which mandates ecotoxicity testing of waters.

Legislation includes ecotoxicity tests (*Daphnia magna)* only in the case of wastewater. The *Daphnia magna* toxicity test is mandatory by the Joint Ministerial Decision 145116/2011 (Determination of measures, procedures and processes for the reuse of treated wastewater) in wastewater reuse applications for municipal wastewater treatment plants that have a population equivalent higher than 100000, and for some classes of industrial effluents. The limit set by this decision is that the toxicity (TU_{50}) of the reclaimed wastewater (before disinfection) to *Daphnia magna* should be lower than 1 (TU_{50}< 1). The frequency of testing is two samples per year.

1.2.6.12 Guatemala

Toxicity testing is not included in any environmental legislation in Guatemala. It is, however, voluntarily applied by a few international companies. Drinking and wastewater quality is determined by physical, chemical and microbiological parameters. Limits for some parameters are quite high. In the absence of law enforcement very few people/agencies comply with the law. Laboratories do not need to have ISO 17025 to carry out any of these tests.

Regarding toxicity testing, all they have for now are ISO or ASTM derived methods to test toxicity of water with Microtox, fresh water and sea water unicellular algae and rotifers (Thamnocephalus). These are voluntary, so they are hoping these will eventually be included in the water and wastewater laws. Proposals based on the Italian legislation have been submitted to the relevant authorities' (personal communication, Pablo Mayorga, 2014) Italy.

According to Dr Renato Baudo (personal communication) effluent is regulated by legislation from different Authorities (EU, State, Regions and Provinces) in Italy. The Italian legislation dealing with water quality (D.Lgs. 152/06), in agreement with the European Water Framework Directive (EWFD), establishes that all types of water discharges, both urban and industrial, including those from water treatment plants, must be licensed by the local Authorities.

All effluents must comply with specified limits of concentration for a number of pollutants. The limits are different for urban effluent discharging in surface water bodies or surface water bodies in sensitive areas, and for industrial effluents, according to the type of production and released pollutants. Local authorities may require limits more stringent than the national prescriptions.

In all cases, water must be returned to the water body from which they were taken with "qualitative characteristics not worse than the original ones and without increasing the hydraulic flow".

Sampling must be made at specified sampling points, immediately upstream of the input into the receiver (surface water, ground water, sea water, sewage system, and, for specific cases, soil and subsoil) either

manually or by automatic samplers. For industrial effluents containing dangerous chemicals the sampling must be done immediately at the factory outlet or its water treatment plant. The Authorities may require the installation of automatic control instrumentation. A sampling protocol, including sample pre-treatment, conservation conditions, and maximum time before analysis, is prescribed. The local Authorities establish a sampling plan, with a specified frequency for the first assessment and a given frequency for the subsequent regular monitoring.

For effluents of urban Water Treatment Plants, controls must be done by the WTP owner (self-control) on an integrated sample (collected in 24 hours) and with a frequency depending on the water treatment plant capacity (with a minimum of 12 samples per year, increased to 24 samples per years for WTP over 50,000 population equivalent; for WTP < 9,999 population equivalent, sampling can be reduced to 4 per years, if the previous controls were all compliant).

The number of allowed non-compliant samples is specified according to the total number of analyzed samples (ranging from 1 for 4-7 samples per year, to 25 for 351-365 samples per year).The Authorities must verify the compliance with specific limits for a number of parameters once per year for WTP < 9,999 population equivalent; 3 times per year for WTP <49,999 population equivalent; 6 times per year for WTP > 49,999 population equivalent.

For industrial effluents, controls must be done on an integrated sample, collected over 3 hours; the Authorities can however do the analyses on integrated samples collected over in a longer period. The 24 hour acute toxicity test with *Daphnia magna* is mandatory. For effluents discharging into surface waters, the sample is not compliant if, at the end of the test, the immobile are ≥ 50 % of the total exposed animals. For effluents discharging into sewage systems, the sample is not compliant if, at the end of the test, the immobility of the total exposed animals is ≥ 80 %.

In addition to the *Daphnia magna* test, others tests may be performed, such as the acute toxicity tests on *Ceriodaphnia dubia*, *Selenastrum capricornutum* (still so called in the Italian law, now re-named *Pseudokirchneriella subcapitata*), bioluminescent bacteria or organisms such as *Artemia salina*, for discharges into salt waters. Other organisms may be suggested in special technical documents prepared in order to update the methods of sampling and analysis.

In case of execution of multiple tests for toxicity, the worst result must be considered. However, a positive toxicity test does not determine the direct application penalties, but only requires further research into the causes of toxicity, and their removal. Standard protocols for the chemical analyses of the required parameters are prescribed.

The main aim is the protection of the quality of the final receiver of the effluents and different approaches according to the different typology of the receivers (surface water, ground water, sea water, sewage system, and, for specific cases, soil and subsoil) are followed.

The effluent dilution (with cooling waters, washing waters, or water strictly used for this aim) to meet the limits is not allowed and explicitly forbidden. The Authorities may even ask that the discharge of cooling water, washing water, or water used for power production, is separated from the factory effluent. Local authorities supervise the verification that the correct application of the procedures for collection, storage, transport and analysis of the samples is implemented.

1.2.6.13 Ireland

The Environmental Protection Agency Act, 1992 (OSPAR 2000) introduced an integrated licensing system for controlling emissions from large/complex and other processes with significant polluting potential known

as Integrated Pollution Control (IPC). Activities covered by the IPC licensing system are listed in the First Schedule of the EPA Act 1992 (OSPAR 2000).

The IPC licences issued by the EPA have, where appropriate, requested acute aquatic toxicity monitoring of effluent emissions which discharge to water or to sewer. The regulatory control of wastewater discharges in Ireland relies on the application of aquatic toxicity monitoring in conjunction with the requirement for testing of the chemical and physical constituents of the wastewater. Compliance with emission limit values for toxicity and other parameters is required and verified by monitoring data submitted by the licensee and also by spot-checks carried out by the EPA (OSPAR, 2000).

When characterising an effluent/wastewater, the licensee is required to undertake an initial toxicity screening test against species from a minimum of four different trophic levels. The licensee must ensure that the tests are undertaken using accepted procedures (ISO, BS, etc.) by a testing laboratory which must be agreed with the EPA. The four trophic levels can be broadly categorised as bacteria, plants or algae, crustacean and fish and there is a list of species which are available for effluent toxicity testing in Ireland. Having identified the most sensitive species, future monitoring is then carried out on the two most sensitive species. In addition to the requirement for toxicity monitoring, the licensee may also have to comply with a toxicity limit expressed in Toxic Units (TU) which also takes into account the dilution available in the receiving system. The number of toxic units is equal to 100/x hour EC/LC_{50} in percentage vol/vol where higher TU values reflect greater levels of toxicity. In most cases, testing is carried out on a 24 hour flow proportional composite sample but where effluent variability occurs it may be necessary to undertake testing on several 24 hour composite samples. The requirement for chronic aquatic toxicity monitoring is assessed on a case by case basis.

Where a wastewater sample is identified as being highly toxic, a Toxicity Identification Evaluation (TIE) or Toxicity Reduction Evaluation (TRE) is employed to identify the likely toxic elements in the wastewater stream and a corrective action programme is put in place to reduce or eliminate the toxicity.

Guidelines for restrictions on the discharge of toxic effluents, expressed in terms of toxicity, are developed on an industry-specific basis. These guidelines are then incorporated on a case-by-case basis in individual permits issued to dischargers. The guidelines recognize the importance of mixing conditions by stipulating that at least a factor of 20 dilutions must be available in the immediate vicinity of a discharge for each toxic unit discharged. Discharge licences in Ireland are based on 96 hour LC_{50} values and dilution is taken into account by applying a dilution factor of 20 in the immediate vicinity of the discharge for each toxic unit (TU is the inverse of the LC_{50}). Different types of industries are given different weightings for acceptance of toxicity (Pedersen et al., 1994). Compliance monitoring is carried out annually or bi-annually on representative samples of effluents. The test species most commonly used is the rainbow trout, *Oncorhynchis mykiss.* Confirmation of the efficacy of toxicity limits is obtained through biological surveys of receiving waters at least once every three years, particularly in areas of biological importance or sensitivity (OECD, 1987).

Although the EPA has not published any data in relation to the various tests undertaken for the sectors covered by the IPC system, all information pertaining to the IPC licences is available for viewing by interested parties at the Agency offices.

1.2.6.14 Japan

In Japan bioassays are not used to date as a monitoring tool. However, a manual of eco-toxicological test methods for chemicals is presently under examination by the Ministry of International Trade, Industry, the Ministry of Agriculture, Forestry and Fisheries and the Environmental Agency. In most cases, the methods proposed are in accordance with OECD Guidelines for the testing of chemicals; that comprise of algal, *Daphnia* and fish tests. Many toxicity methods have already been described in Japanese scientific literature, based on various types of test organisms from different phylogenetic groups such as e.g. bacteria, yeast,

protozoans, micro- and macro algae, crustaceans, molluscs, insects, amphibians, fish and birds (Aoyama et. al., 2000). Management of toxic chemicals in the aquatic environment is performed in Japan by setting environmental standards by law. Forty-nine chemicals are regulated and measured once a month at specific sites in rivers and lakes. Many of these chemicals are detected at various places in Japan, but in concentrations below the standard values. Dioxins, which are not regulated, have been detected in leachates from a landfill area of industrial wastes and also in human mother's milk.

1.2.6.15 Lithuania

In Lithuania, in addition to chemical-based regulation, effluents entering the surface waters have to pass acute *Daphnia magna* test (COHIBA, 2010)

1.2.6.16 Mozambique

The National Water Directorate (DNA) within the Ministry of Public Works and Housing (MOPH) is in charge of overall planning and management of the country's water resources and the provision of water supply and sanitation services in both rural and urban areas. The Water Supply Investment Fund (*Fundo de Investmento e Patrimonio do Abastecimento de Agua, FIPAG*) is a public entity that leases out operations and management to private entities. The Water Regulatory Council (*Conselho de Regulaqzo do Abastecimento de Agua*s) is an independent regulatory agency that sets the tariff regime to ensure a viable and sustainable water sector. As in the case of neighbouring Zimbabwe and Botswana bioassays do not feature in water management legislation and policy.

Regional Water Administrations are basin authorities responsible for water development and management. Mozambique's five Regional Water Administrations control irrigation systems and collect water fees within their jurisdictions. The Regional Water Administrations have administrative, organizational and financial autonomy but report to the DNA (FAO, 2005).

The government body charged with coordinating activities relating to irrigation and drainage is the National Directorate for Agricultural Hydraulics (DNHA) within the Ministry of Agriculture and Rural Development (MADER). The Fund for Agricultural Hydraulics Development has responsibility for promoting and funding agriculture-related water projects (FAO, 2005).

1.2.6.17 Namibia

According to Jürgen Menge, 2014 (personal communication) the primary legislation relating to ownership, allocation, rights to access, and management of the resource is the Water Act 54 of 1956. In addition the National Water Policy White Paper entitled: Policy Framework for Equitable, Efficient, and Sustainable Water Resources Management and Water Services was published in August 2000 by the Ministry of Agriculture, Water and Rural Development.

The "Namibian Water Quality Guidelines and Standards for Potable Water and Effluents" were compiled and updated in 2012. Currently no mention is made to toxicity tests in these standards.

Investigation of the use of whole effluent toxicity tests was initially suggested as part of the initial framework but was not undertaken and subsequently omitted in the present water quality guidelines. The toxicity tests were to be based on the South African DEEEP method to test for deviation from standard as currently used in South Africa And the Greek guideline for total toxicity. Currently the guidelines and standards serve as basis for regulations which will be promulgated, hopefully by end of year.

1.2.6.18 Netherlands

The Netherlands water quality policy distinguishes two approaches: the emission based and the water quality based approach. The emission approach is directed at the assessment of effluents at the source (precautionary principle) and the water quality approach is directed at the effects in receiving waters (Tonkes et al., 1994). Within the Dutch emission policy/approach (also called part A); the assessment of wastewater discharges or effluents is focused on the precautionary principle: the reduction of specific pollutants or substances. Depending on the characteristics and the environmental hazard of a substance, the discharger must remediate a discharge that is known to contain the substance based on BAT or BEP (Best Environmental Practice; see IPPC) with respect to the discharges (Tonkes et al., 1999)

This substance-oriented approach focuses on BAT and further demands are based on certain national criteria (such as maximum permissible risk).

This emission approach has three phases:

- Prevention of pollution.
- Reuse of water and substances where possible.
- End-of-pipe treatment.

Within the third phase (WEA) the same assessment parameters are used as in the second phase, including mutagenicity, acute and chronic toxicity, bioaccumulation, persistence and oxygen demand.

Separate from this, there is a water quality approach, which is based on environmental quality criteria. Finally a stand-still approach is used for new discharges or the extension of existing discharges. The use of WEA might become an extension of this policy Strategy. The possible effects from effluents are only monitored at the end of pipe, and within the process or sewerage systems. The WEA (formerly called WEER, i.e. Whole Effluent Environmental Risk) testing approach including bio-tests is under development. Assessing the biological effects of discharges in the receiving water is not yet practiced in the Netherlands.

Many effluents that occur in the Netherlands are of a complex nature. In the last few decades, numerous measures have been taken to limit surface-water emissions. This has led to an improvement in surface-water quality, but not all water-quality targets have been reached. In addition to certain substance-specific standards being exceeded, biological effects have also been observed in numerous places in the surface water (Hendriks, 1994). For WEA the same assessment parameters are used as for the assessment of specific substances. The WEA method is not meant to predict the effects on the receiving water body, but to complement the assessment of components that are known to be present in a complex effluent (Tonkes et al., 1995).

Comprehensive conceptual work and literature reviews on WEA and some exemplary studies with industrial wastewater among others have been performed. Bio-accumulation was also part of this study. In the most detailed study of Tonkes & Baltus (1997) test results of 10 complex effluents with fish (*Danio rerio*, acute and ”early life stage”), crustaceans (*Daphnia magna,* acute and chronic), algae (*Selenastrum capricornutum)* and bacteria (*Photobacterium phosphoreum*) toxicity tests are reported. Toxkits and Genotoxicity (Mutachrome test with *Salmonella typhimurium*) were also included and effect parameters are tested before and after an additional 28-day degradation step. Moreover, there was an extensive study on cooling water carried out by Baltus, Kerkum and Kienhuis (to be published). At present about 100 effluents discharging into surface waters or sewers have been tested for acute toxicity. Fifty effluents were investigated for genotoxicity and bioaccumulation in cooperation with German institutes. Monitoring surface water toxicity with algae, bacteria, crustacean and fish tests are also reported (Polman and de Zwart, 1994).

The Institute for Inland Water Management and Wastewater Treatment (RIZA) initiated work on the development of effect-oriented methods or techniques in the early 1990’s. The substantial research resulted

in an extensive document has been published in 1994 (“Totaal Effluent Milieubezwaarlijkheid” (Tonkes and Botterweg, 1995).

In 1997 the “Praktijkonderzoek aan complexe effluenten met de Totaal Effluent Milieubezwaarlijkheid (TEM)” was published and was followed in In February 2001 by the “Evaluatie van Project Implementatie Totaal Effluent Toxiciteit (TET). This culminated in a first report on the use of acute toxicity tests for the assessment of complex effluents (Beckers-Maessen, 1994). RIZA is currently developing a method for whole-effluent assessment that considers the following five Parameters (Figure 1-1):

- Acute toxicity: specific short-term, lethal, or potentially lethal effects that occur as a result of exposure to a substance or medium
- Chronic toxicity: specific longer-term, nonlethal effects that occur as a result of exposure to a substance or medium
- Bioaccumulation: the net accumulation of a substance in an organism as a result of combined exposure via direct surroundings and food
- Genotoxicity the ability to cause damage to genetic material or cause an adverse effect in the genome, such as mutation, chromosomal damage, and so on
- Persistence: a substance property indicating how long a substance remains in a certain environment before being converted physically, chemically, or biologically.
- To date the anthropogenic effects from effluents are only monitored at the end of pipes and in the tributary within the process. Attributing the effects in receiving water to the discharge of certain specific effluents is only under debate. If and how this will be done is not yet known. Next to this there is (limited) monitoring, for developmental reasons, of surface waters. Currently this is not related or connected to the effluent policy. It is not related to effluents (OSPAR, 2000).

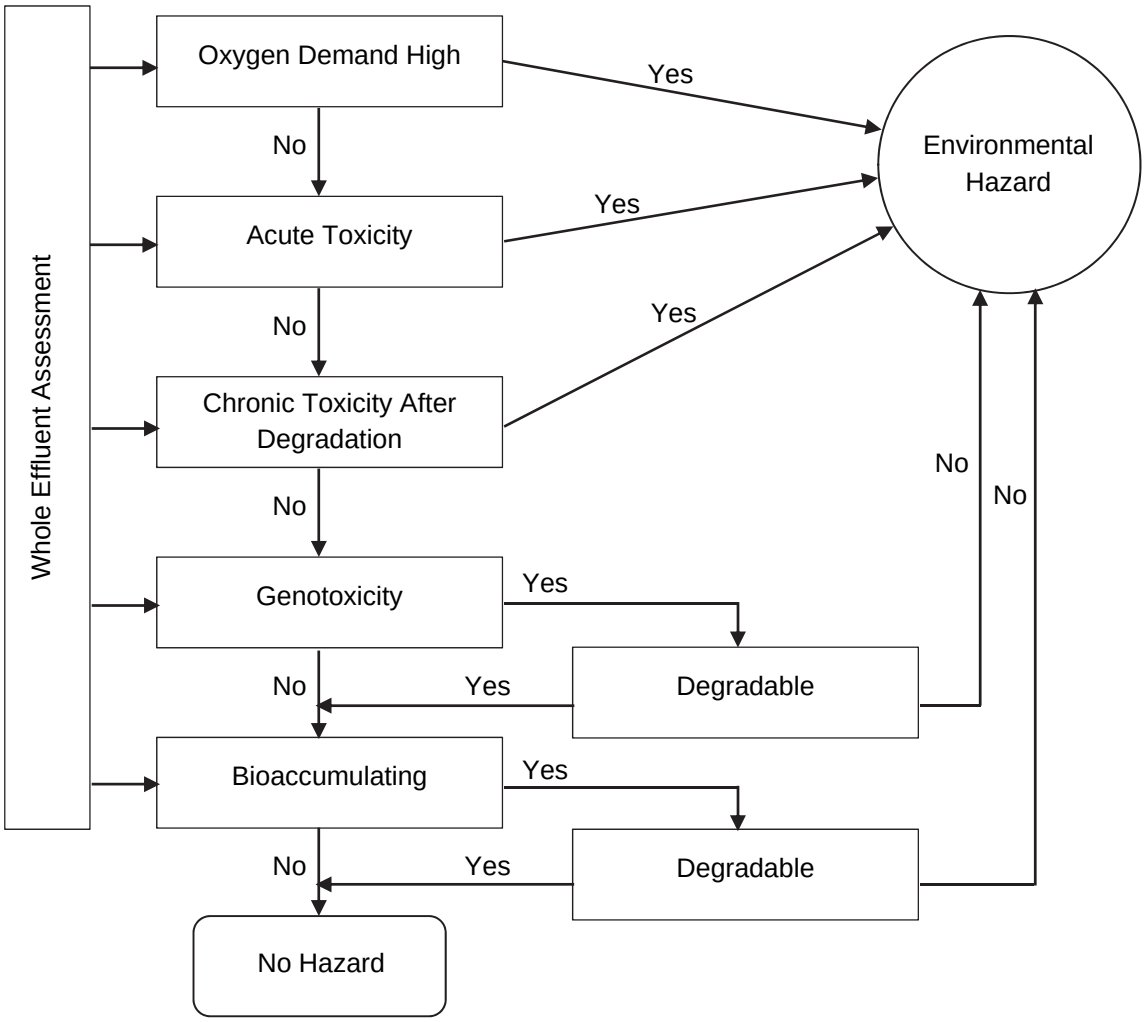

Figure 1-1: Whole effluent assessment in the Netherlands (Tonkes et al., 1995).

1.2.6.19 Norway

WEA, including chemical and eco-toxicological characterisation of effluents is applied on a case by case basis, and used as guidance for issuing discharge permits. Such assessments are normally performed on composite samples of the final effluent from the industry. The chemical analysis programme includes common general water quality and summary parameters as well as specific analysis of selected pollutants. Tests for acute toxicity are performed on algae, crustacean and fish. Marine or freshwater organisms are used depending on the nature of the receiving water. Quantification of potentially bio-accumulative compounds is performed using TLC/GC. Toxicity and bioaccumulation potential may be assessed also after a biological stabilisation of the wastewater performed as a 28 days biodegradation test. For regulation of wastewater emphasis is put on the "total emission of toxicity" expressed as the Toxicity Emission Factor (TEF). In addition a risk assessment is performed on the basis of the toxicity data and predicted recipient concentrations (OSPAR, 2000).

For land-based industry, WEA is used on a case-by-case basis for risk assessments when issuing emission/discharge permits. However, biodegradation, persistency or toxicity values are not used as emission limit values. A WEA guidance document for the authorities will be worked out which might increase a more systematic use of WEA.

For offshore installations eco-toxicological documentation for all production chemicals, drillings fluids and utility chemicals (detergents, hydraulic fluids, etc.) is required by the environmental authorities. All discharges require a discharge permit (OSPAR, 2000). There must be complete documentation of the potential biodegradability and bioaccumulation of the individual organic components in products that consist of several substances. All applications for discharge permits for offshore chemicals and drilling fluids must be accompanied by a HOCNF (harmonised offshore chemical notification format) for the products used in connection with drilling and production, including products used in closed systems. HOCNF and Guidelines for Completing the HOCNF from OSPAR 1995 (OPSAR 2000) must be used. As OSPAR Guidelines for Completing the HOCNF are incomplete according to Norwegian requirements, SKIM (Co-operative forum for Offshore Chemicals, Industry and Environment authorities) has prepared Supplementary Guidelines for Completing HOCNF for the Norwegian sector. The substances shall be tested according to seawater test OECD 306. Other seawater tests that are accepted are *marine CO_2evolution test* (mod. Sturm), *marine BODIS test* (for insoluble substances) and *marine CO_2 headspace test* (mod. ISO N182), which have all been included in PARCOM ring testing (OSPAR 2000). and which give almost the same result. Complete documentation of the bioaccumulation potential of each organic component must be submitted for products that comprise several substances. The substance's bioaccumulation potential must be tested according to OECD method 107 or 117. Offshore chemicals on the Norwegian continental shelf must be tested for toxicity at product level, but SFT will also accept tests at component level, provided that data for all components is given. SFT requires the following three marine toxicity tests:

- *Skeletone macostatum*
- *Acartia tonsa*
- *Corophium volutator* (not required if *Abra alba* has been done) or
- *Scophtalamus maximus.*

When selecting methods, emphasis shall be placed on testing the most relevant species as regards the fate of the product in question (OSPAR, 2000).

1.2.6.20 Poland

According to the Polish law (Dz.U. no 137, item 984) regulation of the Minister of the Environment dated 24 July 2006 on conditions to be fulfilled at the discharge of effluents to water or soil and on substances posing particular threat to aquatic environment (Dz. U. no 137, item 984) with further amendments (Regulation of the Minister of the Environment dated 28 January 2008, Dz. U. no 27 item 169) (COHIBA, 2010).

A new regulation promulgated by the Minister of the Environment concerning classification of surface waters, adjusted to EU legislation, was established in September 2008 (Dz. U. no 162, item 1008) (COHIBA, 2010).

It includes eight groups of indices: physical, aerobic, biogenic, salinity, metals, industrial pollutants, microbiological, and biological. Among the biological indices phytoplankton, phyto-benthos and macrophytes must be analysed. An evaluation of effluent hazards to aquatic environments with biotests is used only in research studies.

1.2.6.21 Portugal

In Portugal there is no legislation on bioassays on effluent monitoring (Brito, 1999) and wastewater monitoring (Morbey and Broto, 1997). Bacteria, algae, and crustaceans (LC_{50} and EC_{50}) are routinely employed for monitoring water quality and occasionally at the outlets in authorisation procedures for special branches of industry focusing on the pulp and paper sector. The Directorate-General for the Environment carries out bioassays on samples collected by the Inspectorate Body in industrial and hospital wastewater treatment plants, and on drinking water supply systems. On the contrary Brito (1999) stated that until 1996 the only bioassay performed was the *Vibrio fischeri* bioluminescence test (OSPAR, 2000).

The National Institute of Industrial Engineering and Technology has carried out two projects. The first project 1990/91 dealt with the development of two tests for the evaluation of acute toxicity for industrial effluents (*Vibrio fischeri, Daphnia magna*). The samples were taken from a surface treatment industry with two ends of the pipe: alkaline and acid discharges. A good correlation between the results of the *Daphnia magna* test and the 5 minute *Vibrio fischeri* test were observed. Nevertheless the correlation between the *Daphnia* sp. and 15 minute *Vibrio fischeri* test was lower (Morbey and Broto, 1997). The second project dealt with the adenylate energy charge in the polychaete *Lanice conchilega*at different sampling points in the vicinity of a cellulose effluent discharge (Morbey and Broto, 1997).

In 1998 the Director-General for the Environment developed a joint project with the Institute of Agronomy in order to evaluate the acute toxicity of pesticides used in paddy fields in the Sado River estuary. Tests were conducted with *Daphnia magna, Thamnocephalus platyurus* and *Rhaphidocelis subcapitata* (freshwater) and *Artemia saline* (saltwater). The project was the first step for the implementation of these tests in routine. In 1999 it was planned to extend the study to the effluents of pulp and paper, tannery, food and pig breeding industries. The objectives were to find a battery of tests for application to the different sectors and to have a scientific study for the elaboration of a legislation framework in the field of eco-toxicological bioassays (Brito, 1999; OSPAR, 2000).

1.2.6.22 Slovenia

The monitoring program of effluents is mainly based on a traditional chemical-specific approach, which involves conventional chemical determinations and measurement of priority pollutants. An assessment of effluent discharging into sewerage systems includes estimation of biodegradability, but toxicity evaluation is not prescribed by regulation. Acute toxicity tests with *Daphnia magna* (according to the ISO-standard) are conducted when toxic substances in the effluents are expected (Tisler, 1999; OSPAR, 2000).

1.2.6.23 Spain

The Spanish monitoring strategy for effluents is directed at the effects for the receiving water bodies and is therefore water quality-based (Tonkes et al., 1995). A special program about organic-fraction toxicity testing has been carried out. For municipal wastes, usually rich in ammonia, nitrites, etc., the specific toxicity testing of the organic (lipophilic) fraction, can be more valuable for the identification of non-expected highly toxic pollutants than Whole Effluent Toxicity Testing (OSPAR, 2000).

1.2.6.24 Sweden

Sweden introduced guidelines for the chemical and biological characterization of effluents in 1970's (Naturvårdsverket, 1989).

These guidelines include whole effluent toxicity assessments and are applied in environmental permits of larger industrial plants. Bioaccumulation and degradability are also analysed. Sweden uses a tiered approach: all discharges undergo the first step of the characterisation, which includes tests on a few organisms from different trophic levels and the emphasis is on acute tests. If the first tier reveals potential adverse effects, the analysis proceeds to second tier and so on (Naturvårdsverket, 1989). The approach is site-specific and takes into account dilution and other properties of the receiving environment. The Swedish EPA is currently updating the characterization guidelines, for example to include endocrine effect testing (Naturvårdsverket, 2010).

Sweden focuses on the prediction of effects by effluents for the receiving water, i.e. the water quality-based approach (Tonkes et al., 1995). Industries have been advised to follow the Characterisation of Industrial

Discharges (CID) guidelines for an evaluation of their effluents and for the supervision and allocation of permits since 1989 (Swedish EPA, 1997).

Therein a combination of biological tests and chemical analyses are recommended to detect substances that are not readily degradable, that are toxic, and/or that bio-accumulate in wastewater. But characterisation according to CID is considered too expensive for small and medium-sized industries (Tarkpea et al., 1998). According to Swedish law a municipal treatment plant has no obligation to accept industrial wastewater. Each municipality can set its own restrictions regarding the substances received into the treatment system. (Tarkpea et al., 1998).

Wastewater is classified as acutely toxic if the concentration after initial dilution exceeds $0,1*EC_{50}$. The Swedish proposal for biotests in WEA includes acute fish and crustacean toxicity, algae as well as tests with higher plants (*Lemna minor, Allium cepa*). A main point is bacteria toxicity which is measured by the activated sludge respiration/nitrification inhibition assay and *Vibrio fischeri* (Swedish EPA, 1997). The detailed environmental hazard and risk assessment scheme was described by Pedersen et al. (1996).

WET test results from different research projects were reported considering short-term algal, bacterial and crustacean tests as well as prolonged biodegradation tests with a modified OECD Screening test (DOC-Elimination) and potentially bio-accumulating substances (PBS).

From 1989 to 1996 an extensive research programme on the characterisation of discharges from the chemical industry (The STORK-project) was carried out. The proposed strategy based on that experience has three successive levels of investigation. Each level takes into account chemical characterisation, degradability, bioaccumulation (BCF > 1 000) and toxicity and the corresponding tools for evaluation. Basic information is compiled from the production process and from previous studies.

- At the first level, COD, BOD7, AOX, TOC, pH, conductivity, P, N, and suspended solids are measured and bio-tests are employed (LC_{50} or EC_{50} for bacteria, higher plants, algae, crustaceans, and fish) in freshwater and saltwater. Investigations must be continued on the next level if no decision can be made concerning changes in the production process, the replacement of chemicals, purification measures and control programmes. In this decision making, technical as well as economic factors have to be considered.
- The second level includes chemical analyses using more advanced techniques (GCMS, HPLC etc.), screening tests on biodegradability, bioaccumulation, and toxicity. Herein the modified OECD screening test (DOC elimination in 28 days according to ISO 7827) and longer-term BOD tests (i.e. 14 days duration) are recommended in Sweden and Denmark (Swedish EPA, 1997; Nyholm, 1996).
- The third level includes a wider range of toxicity tests in cage and field experiments also considering physiological and morphological alterations, population levels and ecosystem/multispecies models (Swedish EPA, 1997). The discharged quantity of toxic substances in effluents is expressed as "Toxicity Emission Factor" (TEF), that is the Toxic Unit (TU) multiplied by the 24hour flow TEF = $[100/LC(EC)_{50}]$*24-hour flow [vol-%*m^3]. Thus a $LC(EC)_{50}$ at 100 volume present and a flow of 100 m^3/d corresponds to 100 TEF units. TEF values lower than 100 are deemed acceptable (Swedish EPA, 1997; OSPAR, 2000).

1.2.6.25 Switzerland

The assessment of effluent in Switzerland is focused on the effects on receiving water bodies Standard requirements, such as fish toxicity and non-disturbance of the biological purification process are considered. Also differentiation of limits is made between discharges into sewers and those discharged directly into surface waters (Tonkes et al., 1995). There are no regulations concerning the eco-toxicology of wastewater effluents. Within research projects genotoxicity of hospital wastewater was evaluated with the UMU C-test and a genotoxicity identification evaluation confirmed that fluoro-quinolone antibiotics cause genotoxic

effects in hospital wastewater (Giuliani et al., 1996; Hartmann et al., 1998). No further information regarding WET testing is available (OSPAR, 2000).

1.2.6.26 United Kingdom

During the 1990's The United Kingdom (UK) water quality management policy required that quality of receiving watercourses be considered, i.e. the 'water quality approach'. Environmental quality standards (EQS) were set to protect the ecosystem and maintain the quality for specific use, taking into account dilution and dispersion (Tonkes et al., 1995). There were no regulations stipulating eco-toxicity testing for effluents on a national basis, but bioassays were occasionally used in compliance monitoring. There were approximately 20 toxicity-based consents in place (Tonkes et al., 1995), but these were reviewed and standardised once appropriate guidelines were developed. Recommendations were made to include Direct Toxicity Assessment (DTA) along with chemical-specific assessment in the evaluation of effluents and a demonstration project was launched to assess the use of DTA in a regulatory context. This led to the phased and consistent introduction of DTA controls to appropriate discharges (OSPAR, 2010).

A scheme for the biological monitoring and control of point source discharges was subsequently drawn up. Three standard acute toxicity tests using species from three taxonomic groups, namely algae, invertebrates and fish will be used to derive toxic based consents. A luminescent bacterial test was to be calibrated against the most sensitive of the three test species to provide a simple and relatively inexpensive test for routine use (OECD, 1987; Hunt et al.,1992).

Prior to 2000 research and development received substantial attention and great efforts have been made to develop and establish many new test systems which are described in detail in various Research and Development Reports (e.g. The United Kingdom Environment Agency (UK EA), 1998 and 1999). Direct Toxicity Assessment (DTA) for instance has been used widely in the context of research, development and demonstration, and numerous projects have been completed to support the use of DTA to monitor and control effluents (OSPAR, 2000): These include projects to:

- Develop and evaluate methods e.g**.** *Daphnia magna* reproduction test, enhanced chemo-luminescence (ECL),chlorophyll fluorescence, marine bacterial luminescence, aquatic invertebrate fluorescence (UK EA, 1999c, 1998c & 1999d, 1999a,1998b, & 1999e).
- Improve and standardise methods for example using Image Analysis in the Oyster Embryo-Larval Development and *Daphnia magna* growth tests (UK EA, 1999), ring-testing the OECD *Lemna* growth inhibition and the 48h *Tisbe battagliai* lethality test (UK EA, 1999f & 1999g) producing method guidelines for effluent and receiving water assessment (UK EA, 1999h), Standardising procedures for the Microtox® test system: Acute, Chronic, Solid-phase and Mutatox® (UK EA, 1999).
    - Develop quality control and assurance procedures for example: Developing a proposed scheme to ensure the quality of data generated by laboratories undertaking regulatory eco-toxicological testing (UK EA, 1999b) and developing performance standards for eco-toxicity tests.
- Improve the way in which eco-toxicity test data are used in risk assessment, e.g. Statistical analysis of effluent bioassays, (UK EA, 1998a), the analysis and use of limit tests (UK EA,1997) and developing a risk framework for direct toxicity assessment of effluent discharges (UK EA, 1999).
- Demonstrate the use of the tests in the management of effluents e.g**.** Toxicity Based Consents – Pilot Study, (UK EA, 1996a), the direct toxicity assessment demonstration programme, (UK EA, ongoing work) and toxicity reduction evaluation case summaries for the pulp and paper industry and the chlor-alkali industry, (UK EA, 1996b and 1996c).

Research and development was also undertaken to investigate and demonstrate the benefits of using DTA in assessing effluent and extensively recorded (Weger 1993; Lewis et al., 1994; Boumphrey et al., 1999).

Nationally (UKEA) and internationally (OECD) standardised acute toxicity tests with fish (*Oncorhynchus mykiss, Cyprinus carpio*), acute and chronic test *with Daphnia magna* and tests with algae (*Selenastrum, Skeletonema*), *Vibrio fischeri* and various other organisms (oyster embryo larval, *Tisbe battagliai*, *Acartiatonsa*, *Gammarus pulex* and *Lemna minor*) have been used in research and development projects (UK EA, 1996a & b).

Sampling takes place at a point appropriate to the objectives of the testing. It is proposed that routine regulatory testing would take place at the end of pipe, but the way in which the result would be interpreted and used will take account of the dilution available in the receiving water, and other receiving water characteristics. During the characterisation of the effluent, sampling may take place at many different places, e.g. at the end of pipe, at a point in the receiving water, or up and down stream of the discharge outlet in order to see how the toxicity in the water changes (UK EA DTA Demonstration Programme, in progress). If unacceptable toxicity is found in the effluent, sampling will take place further up in the process to determine the sources and causes of the toxicity. The numerous test results available (LC_{50} and NOEC in % effluent) are published in detail in *Toxicity Based Consents Pilot Study* (UK EA 1996a).

The UK has developed a seven stage protocol for assessing and regulating effluents (Forrow, 1999). This protocol has been derived as a result of previous research and development (e.g. National Rivers Authority, 1993) and public consultation, and is tested in the 'DTA Demonstration Programme'. This programme is a collaboration between the UK regulators, industry and water companies.

The protocol enables the regulator to prioritise resources, and investigate and manage complex effluents. The first stage of the protocol directs the investigation towards receiving waters where the biological quality of the aquatic system is already impaired (i.e. the existing 'worst cases'), and where there is a likelihood that this is due to toxic substances (as opposed to, for example, oxygen depletion).The effluents are then characterised using a range of toxicity tests, a risk assessment is made and a level of toxicity is derived at which 'no harm' is thought to occur in the receiving water. If unacceptable toxicity is found, a site and process audit and TIE would be undertaken, and a toxicity reduction programme derived. This would be assessed using BAT criteria and a plan for implementation, with associated timescales, would be put forward to the regulator. The plan would be implemented, and the success of the programme in terms of toxicity reduction and changes in the receiving environment appraised and fed back into the management process.

This protocol (Figure 1-2) is only one approach for using DTA in effluent management, whilst it targets priority locations, it does not consider other objectives, e.g.

- where a new discharge is to come on line, or where a substantial change to the process occurs which is likely to result in an increase in toxic emissions;
- concerns where there are vulnerable/sensitive environments (e.g. habitats of special interest, fisheries);
- environments where biological survey is difficult or not possible (e.g. marine, estuarine, constructed environments);
- moves to more precautionary approaches (e.g. to prevent harm rather than react to its occurrence);
- where DTA could be used in determining process or treatment BAT.
- where voluntary activity by industry should be encouraged to: investigate and reduce risks to the environment; protect treatment plants from toxicity.

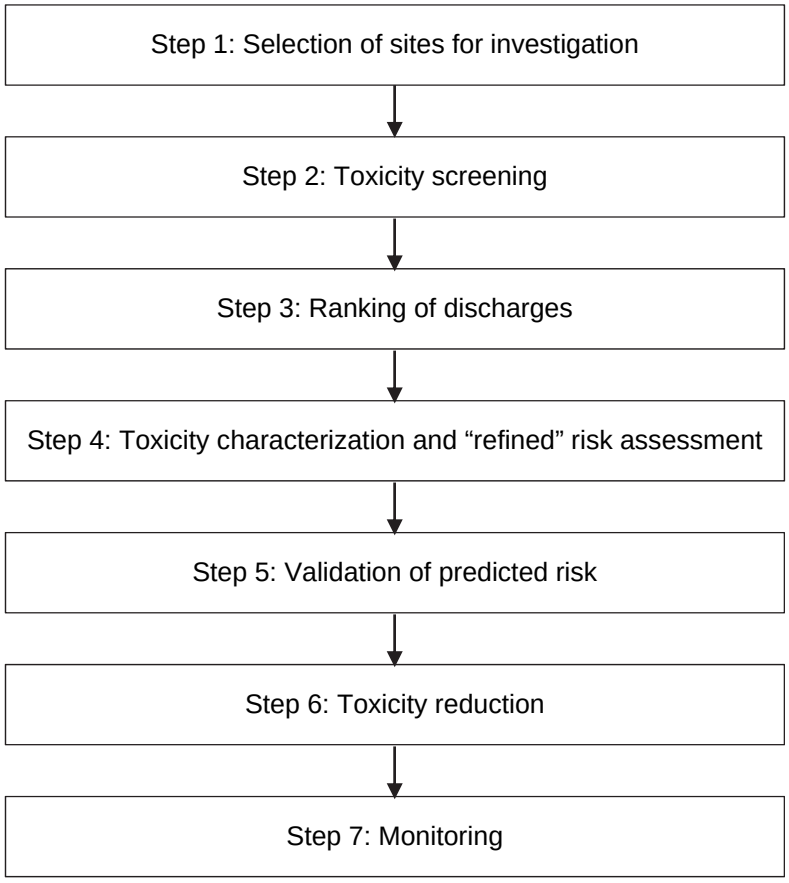

Figure 1-2: Seven stage protocol developed in the UK (Den Besten and Munawar, 2005).

Whilst the UK has not undertaken any practical demonstration in these other areas, they are considering approaches for how best to use DTA in these situations (UK EA, 1999).

The UK approach focuses on the three levels end of the pipe, toxicity close to the outlet, and changes of the ecosystem related to toxicity and other anthropogenic effects. The starting point however is not the toxicity of the effluent, but the quality status of the receiving water, which is determined in ecological monitoring studies. The UK protocol for monitoring and control of discharges from point sources, (from Tinsley, 1999) is shown in Annex V-6.

The UK DTA approach for the management of discharges from point sources focuses primarily on the quality of receiving waters for the following reasons:

- A risk based approach, taking into account the quality of the receiving water, has been demonstrated to be an effective way to prioritise limited resources.
- Environmental conditions may change the nature of the effluent (for better or worse), and so should be taken into consideration in a risk assessment.
- The risk-based approach, which takes account of receiving water dilution and quality, allows cost and benefit to be considered, as is necessary to determine BAT.
- BAT will not be achieved if the toxicity of the discharge is not balanced with other BAT criteria such as the need to minimise waste and the use of resources (e.g. water use).
- BAT will not be achieved if over emphasis is given to the toxicity of the effluent, with no consideration of environmental capacity (Boumphrey et al, 1999).

Development of DTA is on-going, and toxicity assessment methods which will better predict the effects of continuous low level exposures of chemical mixtures on populations of organisms as well as *in-situ* receiving water and rapid toxicity assessment methods are being developed and tested. Toxicity limits will not be

applied in an industry sector by sector basis, but in a site-specific, case by case basis, taking into account the needs of the receiving water environment (OSPAR, 2000).

This would be assessed using BAT criteria; a plan for implementation, with associated timescales, would be put forward to the regulator. The plan would be implemented, and the success of the program, in terms of toxicity reduction and changes in the receiving environment, appraised and fed back into the management process.

The UK approach focuses on three levels:

- End of pipe
- Toxicity close to the outlet
- Changes of the ecosystem related to toxicity and other anthropogenic effects

Most recently (Leverett, 2003), the UK EA has prioritized a number of industrial effluents based on intrinsic hazard (measured toxicity). The final ranking of these effluents will eventually also account for the environmental risk (volume of discharge, dilution in the receiving environment, flows, tides, and so on). Once complete, this will allow the focusing of resources on the control and remediation of effluents with the potential to cause most toxicity problems in the environment.

1.2.6.27 United States of America

The Federal Water Pollution Control Act of 1972, as amended by the Clean Water Act (CWA) of 1977 and by the Water Quality Act of 1987 (USA, 1987), specifies the objectives of restoring and maintaining the chemical, physical, and biological integrity of the nation's waters. Protection of aquatic life and human health from impacts caused by the release of toxicants to surface waters is called for by the Act, which states that "it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited'".

The National Pollutant Discharge Elimination System (NPDES) permit programme, mandated by the Act, regulates the discharge of pollutants from point sources. In order to assess and control the discharge of toxic substances through the NPDES permit programme, the United States Environmental Protection Agency (US EPA) has issued a national policy statement entitled "Development of Water Quality-Based Permit Limitations for Toxic Pollutants" in 1984. The policy supports an integrated strategy consisting of both chemical and biological methods to address toxic and non-conventional pollutants from industrial and municipal sources. The EPA's *surface toxics control* regulation, issued on 2 June 1989 (US EPA, 1989), established specific requirements that an integrated approach be used in *water quality-based toxics control.* For the protection of aquatic life, the integrated approach consists of whole effluent and chemical-specific testing. As techniques are made available for implementing bio-criteria (direct measure of ambient aquatic life and overall biological integrity of a water body), they too will be integrated into the water quality-based toxics control. Each approach has its limitations and for this reason exclusive use of one approach alone cannot ensure the required protection. For the protection of human health, technical constraints do not yet allow for full reliance on an integrated strategy, and thus primarily chemical-specific assessment and control techniques are employed (US EPA, 1991a). The integrated approach to water *quality-based toxics control* relies on the water quality standards that each State has adopted. All States have water quality standards consisting of both chemical-specific numerical norms for individual pollutants, and narrative "free from toxics in toxic amounts" criteria. The use of toxicity testing and whole effluent toxicity limits is based on a State's narrative water quality criterion and/or in some cases, a State numeric criterion for toxicity (US EPA, 1991a).

The whole effluent toxicity testing approach for the protection of aquatic life involves the use of acute and chronic toxicity tests to measure the toxicity of wastewaters. The EPA has published extensive written protocols listing numerous plant, invertebrate and vertebrate species for toxicity testing (US EPA, 1991b, c). At various points during testing the number of organisms affected is counted and the lowest effluent

concentration that causes an effect is calculated. This concentration, referred to as the endpoint concentration, becomes a quantified measure of the concentration that would cause in stream impact if exceeded for a particular period of time (US EPA, 1991a). It is usually stated either as an LC_{50} (the concentration at which 50% of the test organisms are killed) or a No Observed Effect Concentration (NOEC) (the highest effluent concentration at which no unacceptable effect will occur even at continuous exposure). The toxicity measurement can then be used to limit the discharge of toxicants in an effluent. Toxicity itself is used as the effluent parameter. The toxicants creating that toxicity need not be specifically identified or controlled where the effluent's toxicity is limited. Acute (TUJ and chronic (TUc) toxicity units are used as a mechanism for quantifying in stream toxicity when using the whole effluent approach. The number of toxic units in an effluent is defined as follows:

- $TUa = 100/LC_{50}$, and
- TUc = 100/NOEC,
- where 100 = the whole effluent toxicity (no dilution) expressed as percentage (100%) and
- both LC_{50} and NOEC are calculated as percentage dilution of the whole effluent.

The procedure to implement the narrative criteria using a whole effluent approach should specify the testing procedure, the duration of the tests (acute or chronic), the test species, and the frequency of testing (US EPA, 1991a).

In the United States, DTA, or WET testing as it is called, is thus considered to be an integral part of a three-pronged approach to the control of toxic chemicals in waterways (US EPA 1991b). These involve:

- chemical-specific guidelines and measurements;
- WET testing; and
- Field bio-assessment.

The development of WET testing in the United States has been accompanied by the development of standard protocols for appropriate acute and short-term chronic tests in fresh and salt water (Klemm et al. 1994; Chapman et al., 1995, Heber et al. 1996). Since the 1980s, acute and chronic toxicity limits have been incorporated in wastewater discharge permits of industrial and municipal treatment facilities. The test methods vary geographically. More recently the US EPA (1995) has required that all major industrial and municipal discharges undergo a potential analysis for WET testing, using manuals for acute, chronic freshwater, and chronic saltwater protocols. These test protocols have been assessed in a series of intra-laboratory and inter-laboratory comparisons (De Graeve et al., 1991, 1992), and they showed routine success. Similar methods to these are used as a basis for deriving water quality guidelines.

Figure 1-3 provides an overview of water-quality-based “standards to permits” process for toxics control.

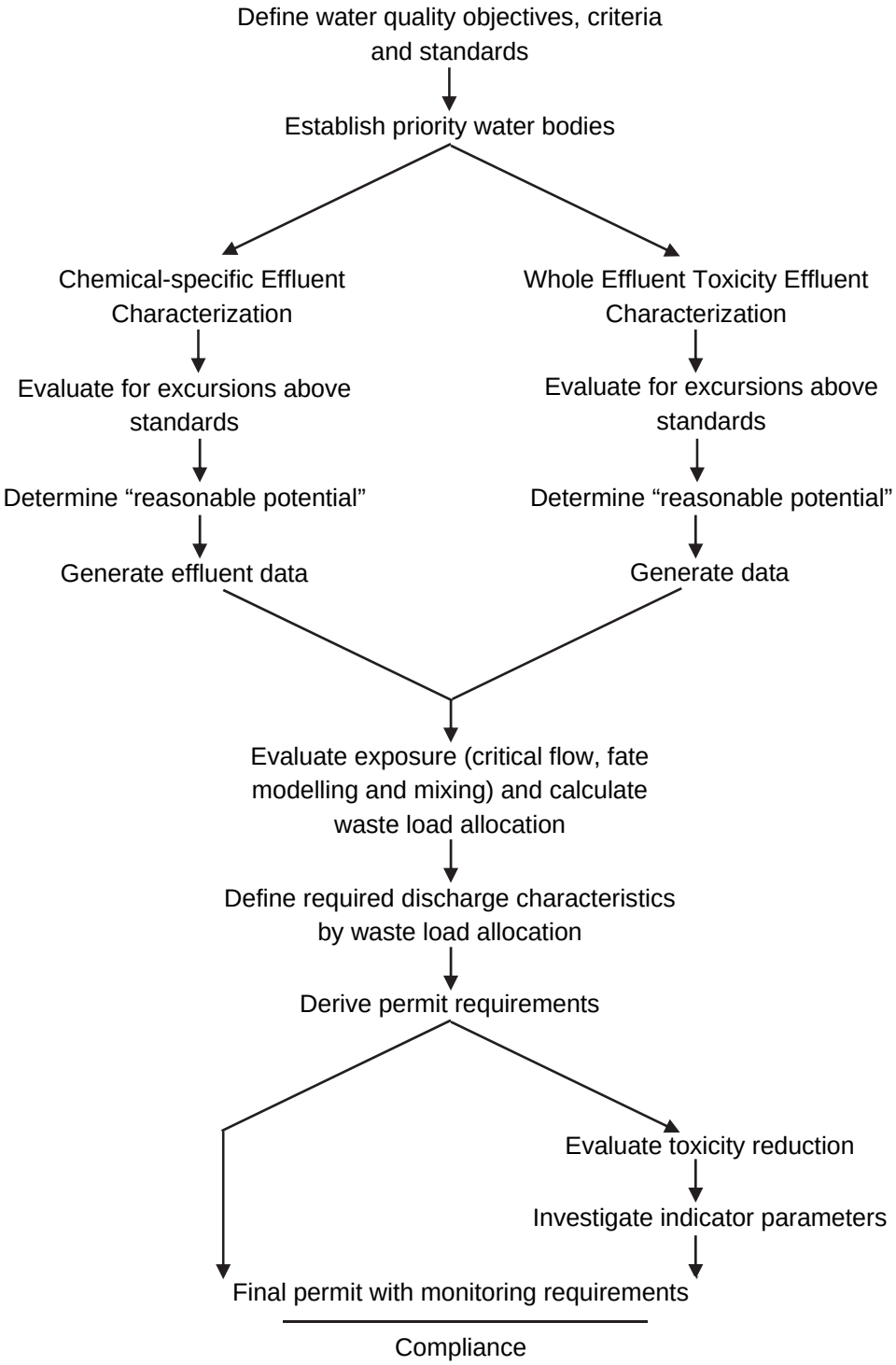

Figure 1-3: Overview of water-quality-based “standards to permits” process for toxics control.

The USA is believed to be the most progressive country outside Europe as far as the prescription of toxicity requirements in discharge permits is concerned. Many states have legally based toxicity requirements (Tonkes, 1994). WET testing has an important role in US-EPA´s water quality program. Most industries are regulated by effluent guidelines based on the best available technology incorporating economic considerations. Heber et al. (1996) reported that at that stage over 6500 effluent permits include WET monitoring or WET limits on a case by case basis. The WEA Guidelines developed by the U.S. Environmental Protection Agency (EPA) were published in three detailed technical documents available on the Internet. There, test methods, ecological relevance and culturing conditions as well as statistical data analysis are described:

- Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth edition EPA/600/4-90/027F (Webber, 1993).
- Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater. Fourth edition EPA/600/4-91/002 (Lewis et al., 1994).
- Short-term methods for estimating the chronic toxicity of effluents and receiving waters marine and estuarine organisms. Fourth edition EPA/600/4-90/027F (Lewis et al., 1994).

The Clean Water Act and EPA regulations authorise and require the use of an integrated strategy to achieve and maintain water quality standards, considering chemical-specific analysis, biosurveys in the receiving water, and WET. The WET program gives a characterisation of the whole toxicity of an effluent without necessarily knowing all of its components and considering the effects of bio-available substances. The strategy is completed with toxicity reduction evaluations (TRE's) and toxicity identification evaluations (TIE's) (Huwer and Britz, 1999) in order to identify and reduce pollutants at the source (Tonkes et al., 1995).

There are guidelines for conducting toxicity identification/reduction evaluations of toxic effluents using BAT. The US EPA recommends that the method used in any given wastewater evaluation exercise should be the method giving the highest degree of protection. The starting point for determining which wastewater investigation should be carried out is a calculation of the dilution capacity of the recipient (the mixing zone). A potential dilution factor greater than 1 000 at the minimum water flow leads to the recommendation of three types of acute toxicity tests (plant, invertebrate, vertebrate). The evaluation should enable one to set a CMC (Criteria Maximum Concentration, defined as 0,3 times the lowest LC_{50} value). For a dilution factor between 100 and 1 000 at minimum water flow either acute or chronic toxicity testing is recommended to calculate a CCC (Criteria Continuous Concentration). A factor below 100 indicates the recommendation of chronic tests for CCC calculation. For unacceptable toxic effluents the local authorities are entitled to demand a TIR or TRE. Principles for investigating and assessing the environmental risks of industrial wastewater were initially developed and implemented in the USA (US EPA, 1991a).

Figure 1-4 gives an overview of a workshop held in Pellston, MI, in 1995 that focused on the science of WET testing. Grothe provided a state-of-the-art overview (current at the time) of the following topics:

- The appropriateness of the endpoints used in routine WET methods
- The degree and causes of method variability in WET testing
- Biotic and abiotic factors that can influence measured field responses to effluents
- The relationship between effluent toxicity, ambient toxicity, and receiving-system impacts.

The Clean Water Act and EPA regulations authorise and require the use of an integrated strategy to achieve and maintain water quality standards considering chemical-specific analysis, bio-surveys in the receiving water and WET. The WET-program gives a characterisation of the whole toxicity of an effluent without necessarily knowing all of its components and considering the effects of bio-available substances. The strategy is completed with TIE and/or TRE (Huwer and Brils, 1999) in order to identify and reduce pollutants at the source (Tonkes et al.,1995).

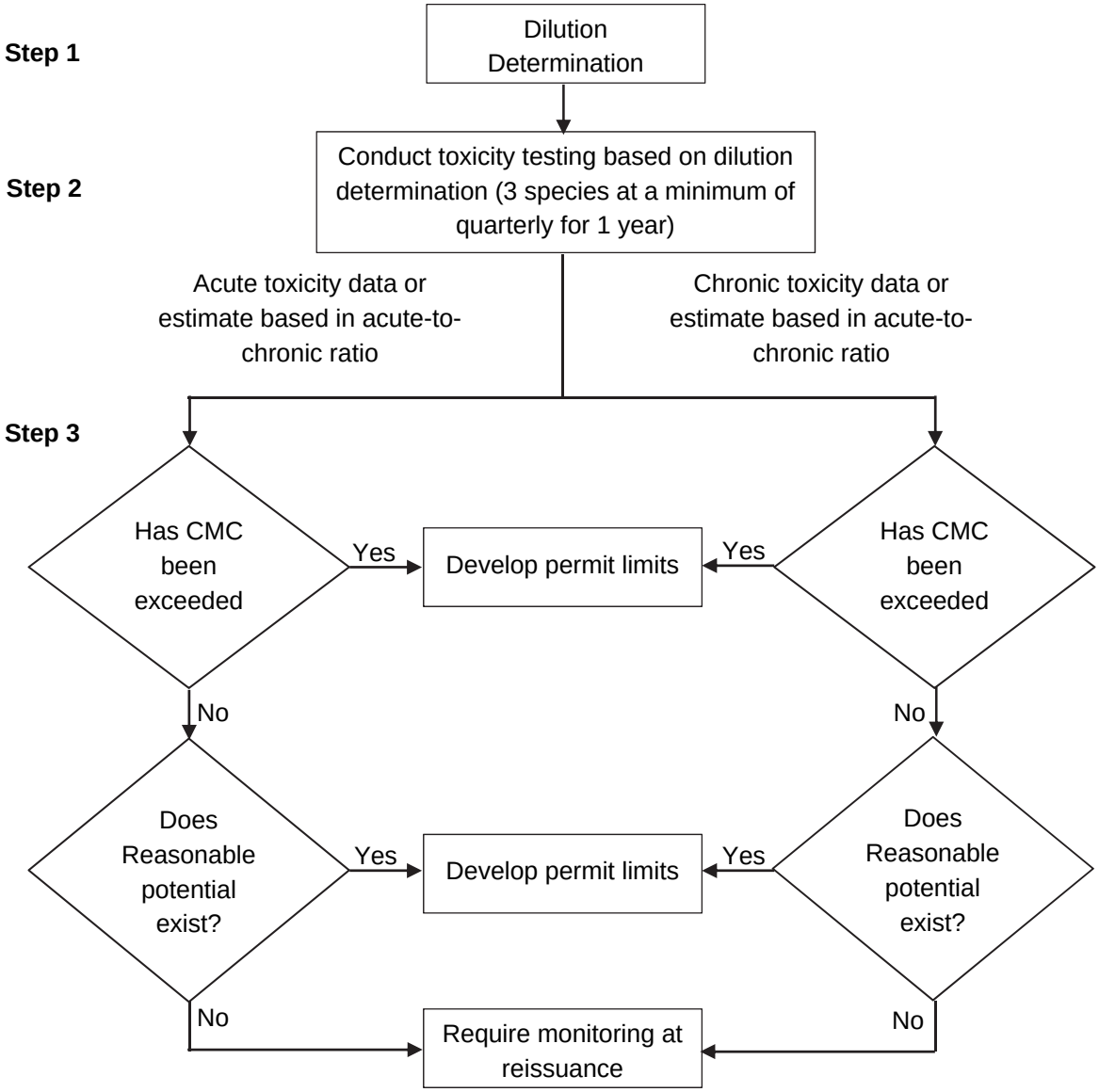

Figure 1-4: Effluent characterisation for whole effluent assessment (Grothe et al. (1996).

1.2.6.28 Zimbabwe

The current legislation focuses on the implementation of water resource allocation and control and does not refer to toxicity testing and water quality issues. Efforts towards pollution control have intensified, with the "user pays" principle being adopted. Water management functions have also been decentralized to the catchment or watershed scale where stakeholders have a say in the management of water in their own areas. The Zimbabwean National Water Act (1998) governs the use of water in Zimbabwe and was signed into law after considerable consultation with stakeholders. The new act is founded on economic efficiency, environmental sustainability and equity of use. For the purpose of this study the following are important features:

- Water rights have been replaced with water use permits. The permits are issued for a limited period and can only be renewed subject to water availability and evidence of efficient use.
- There is greater consideration of the environment, with environmental water use now recognized as a legitimate user.
- Water management has been decentralized to stakeholder-managed Catchment Councils (CCs) and Sub-Catchment Councils (SCCs).

- The Zimbabwean Water Act (1998) has also paved the way for better institutional coordination to facilitate more efficient water management.

1.2.7 SOUTH AFRICA

1.2.7.1 Legislation and Policy

South Africa National Water Act (Act 36 of 1998)

Chapter 3 of the South Africa National Water Act (Act 36 of 1998) (SA NWA) is aimed at protecting water resources and provides for water in sufficient quantity and in sufficient quality for basic human needs and for maintenance of aquatic ecosystems. As the 30th most water scarce country in the world South Africa's limited water resources comprise inland surface water, water courses (rivers, springs, natural channels, wetlands, lakes and dams), estuaries and aquifers. To reduce and prevent degradation of these water resources and to assess its quality, the Act (RSA, 1998) mandated the establishment of a national monitoring system. To achieve this objective the discharge of effluents into water bodies has to be managed and the main criteria for assessing whether effluents could be discharged is primarily based on substance specific guidelines. In accordance with international trends it was accepted that an ecological effect-based approach should supplement substance specific management practices (DWA, 2003)

The use of regular ecological bioassays of receiving water resources as well as complex industrial wastewaters (effluents) which are released into these waters thus featured prominently in the following inter-dependent and complementary resource- and source directed strategies:

- The National Toxicity Monitoring Programme (NTMP) for water resource management (DWAF, 2005) as well as
- the Direct Estimation of the Ecological Effect Potential (DEEEP) approach to manage source directed control or more specifically effluent discharge into surface waters (DWA, 2003).

The use of aquatic toxicity tests widely applied in both the resource directed- and the source directed measure but this project will focus only on the use of aquatic toxicity bioassays as tool to monitor discharge of final effluent into the receiving waters.

Integrated Water Resource Management (IWRM)

In spite of substantial changes in environmental legislation in South Africa over the past decade, water resources regulation is still suspect as is evident by high levels of illegal water use and deteriorating water quality. There is growing public concern regarding the state of South Africa’s water resources one major reason being major regulatory capacity (Schreiner et al., 2011).

The responsibility for ensuring quality water is not confined to the Department of Water and Sanitation (DWS). There are several government (provincial and local) departments and non-governmental agencies which are charged to enforce measures to enhance water quality. According to Schreiner et al. (2011) it is imperative that direct water resource regulatory institutions and policies and procedures by such institutions are aligned. This should apply to other relevant regulatory institutions such as for land use, environmental regulation and natural resources. According to Schreiner et al., (2011) effective regulation of the water sector should be a critical focus of the Department of Water and Sanitation.

The national Department of Environmental Affairs has considerably improved its capacity for compliance monitoring and enforcement of regulation. However, environmental management is constitutionally a concurrent competency and there have been wide-spread concerns about the capacity of provincial departments to implement the legislation effectively, particularly in relation to the delays in the processing of environmental impact assessments (EIA’s) and Water Use Licences (WUL).

The Department of Environmental Affairs has established a unit called the Green Scorpions which are responsible for the enforcement of environmental legislation. The Department is also involved in negotiations with the Department of Justice to open four environmental courts in Gauteng, Mpumalanga, the Western Cape and the Eastern Cape. These will be normal courts in which a certain amount of time will be set aside for addressing environmental cases, including water cases. The Department has already been training prosecutors and building awareness amongst magistrates about environmental legislation and have compiled both a compendium of environmental legislation and a prosecutors' manual

While the water services regulatory strategy has thus been fully developed, a comprehensive regulatory strategy for water resources management is still required. This should focus on the overall regulation of water resources as a critical part of water resources management. A particular challenge is to develop a regulatory framework which will suit the needs of a developing country (Figure 1-5). The water management system is presently highly centralized with only two Catchment Management Authorities (CMA's) operating with limited delegated authority. Internationally a decentralised model is favoured, largely due to the improved responsiveness to local conditions and participation at grass roots level. The scope responsibilities and assignment of authority to CMA's is presently under review and in this regard it is imperative that consideration be given to the process of granting WUL as this might involve major policy changes (capacity, training and guidelines). This project is focussed on providing an expert and contemporary system to facilitate the applications and providing relevant guidance in respect of bioassays to be used in this regard.

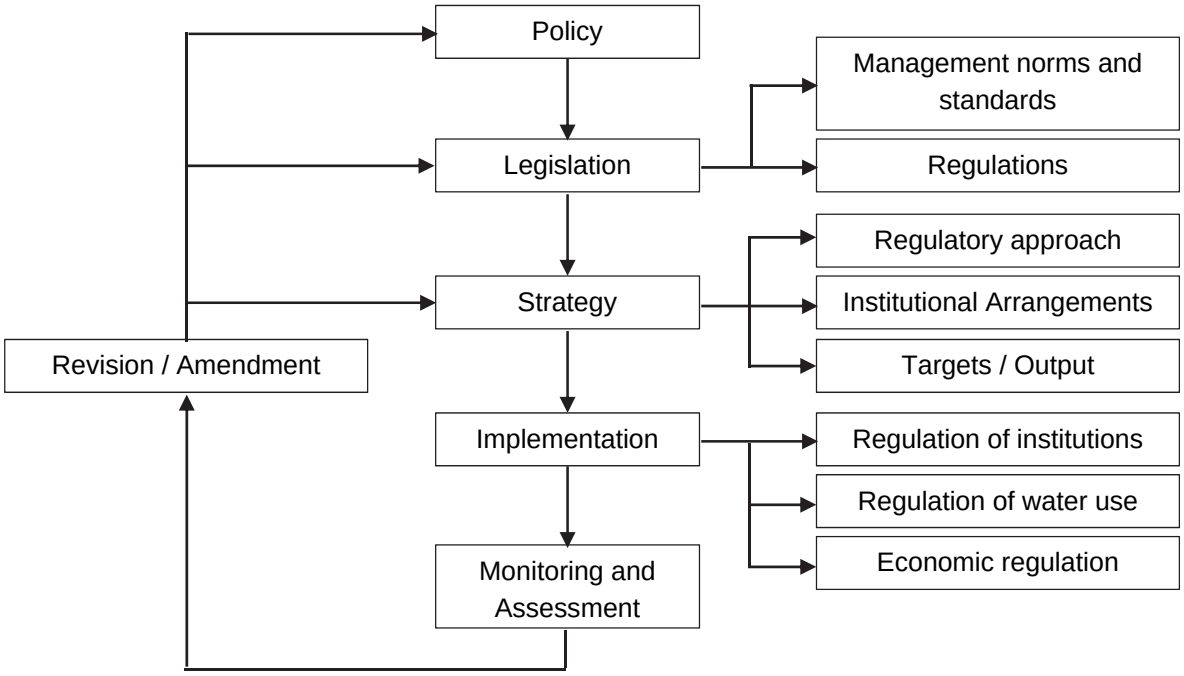

Figure 1-5: Key functions in Water resource regulation (Schreiner et al., 2011).

Integrated Water and Waste Management Plan (IWWMP)

Source document "Operational Guideline: Integrated Water and Waste Management Plan for the preparation of the Water Quality Management Technical Document to support the Application for Licences for Mining and Industries in Terms of the Requirements of the South African National Water Act, Act 36 of 1998 (RSA, 1998).

The globally accepted principles of integrated catchment management prescribe that pollution-related issues should be addressed by approaching conservation, management and use of water resources in a holistic manner. The Integrated Water and Waste Management Plan (IWWMP) has therefore been compiled to support mines and industries applying/renewing for Water Use Licences (WUL) in terms of section 40 (1) of the National Water Act, 1998 (Act 36 of 1998). The aim of the IWWMP is to assist the users in compiling water quality management technical documents in accordance with an approach acceptable to all the

stakeholders concerned. In addition the IWWMP aims to assist in the motivation of the application as well as to assist the decision makers whether to approve the application or not (DWA, 2010). The practical value of this quality and user-friendly guidelines is illustrated below:

Although the requirement for the compilation of an IWWMP was originally aimed at collating and rationalising the information submitted for water use licence applications according to the Operational Guideline, it has progressed beyond this to:

- Provide the regulatory authorities with focused and structured information not only to meet their general information needs, but also to articulate the required management measures and actions to achieve the water and waste related performance on an on-going basis;
- Provide direction and guidance to the water user on water and waste management of any activity.

According to the IWWMP Operational Guideline the additional purpose of an IWWMP is as follows:

- Compilation of a site specific, implementable, management plan addressing all the identified water use and waste management related aspects (e.g. process water balances, storm water management, groundwater management, water reuse and reclamation, water conservation and demand management, waste minimization and recycling) of a specific activity, in order to meet set goals and objectives, in accordance with IWRM principles;
- Provision of a management plan to guide a water user regarding the water and waste related measures which must be implemented on site in a progressive, structured manner in the short, medium and long term; and
- Documentation of all the relevant information, as specified in this guideline, to enable DWA to make the decision regarding the authorisation of a water use.
- Clarification of the content of the IWWMP for DWA officials and the water users, as the various regional offices of DWA might have different interpretations regarding the content of an IWWMP.
- Standardisation of the format of the supporting documentation which DWA requires during submission of a Water Use Licence Application (WULA);
- Provision of guidance on the content of information required in an IWWMP as part of the water use authorisation process and level of detail that DWA requires to enable them to evaluate the supporting documentation to make a decision on authorising water use;
- Ensuring that a consistent approach is adopted by DWA and the various Regional Offices and CMA's with regards to IWWMPs.

The Operational Guideline document for Integrated Water and Waste Management Plan as published by DWA (2010) was intended to be used by the following target audience:

- Departmental officials, inclusive of Regional Offices and Catchment Management Agencies who are directly involved in the Department of Water Affairs' water use authorisation process; and
- Consultants, interested and affected parties, water users, mines and industries that require an understanding of the technical requirements of the DWA for water use authorization.

The IWWMP must supply DWA with a very clear management commitment to ensure the implementation of the IWWMP action plan. The completed IWWMP needs to be endorsed and implemented by the water user, and it will be captured as a water use licence condition. The implementation of an IWWMP is therefore enforced through the water use licence conditions in the water use authorisation process. Effect assessments could be applied within a law enforcement context, namely to set standards used in source directed controls. Effect-based assessment could also be applied to elicit a site- or situation specific response to a stressor. This will be required where objectives are set in resource directed measures. Each of these applications have a different set of test requirements with reference to precision, test organism/test material choice, exposure time, etc.

The action plan of the IWWMP will be subject to an annual review as stipulated in the WUL based on the integrated water resource management principles of continual improvement throughout the entire life cycle of the activity. Comment from DWA can be addressed as part of the annual review of the IWWMP action plan. This requirement would be enforced through the water use authorisation process and the water use licence issued for the activity. The review process will allow DWS to monitor the performance of the activity in terms of the impact on the water resource and the effectiveness of the water use and waste management measures stipulated in the action which needs to be implemented to achieve the set objectives.

As indicated in the original IWWMP Operational Guideline documents, it should be used in conjunction with other guidelines as developed by DWS, such as the External Guideline on the Water Use Authorisation Process and the series of Best Practice Guidelines for water resource protection in the Industries and Mines. Furthermore it advocates that the IWWMP is a living document that needs to be updated and "kept alive" as new information becomes available to provide on-going and updated guidance to the water user on their water and waste management.

In contrast, and in the absence of clear guidelines there is not such a consistent and uniform approach by the various DWS regional offices towards the development and requirements relating ecological bioassays. Departmental officials or water users are subjected to personal interpretation of what information should be contained in a water use licences applications.

In summary it could be stated that the DWS requires an IWWMP (Figure 1-6) as a simple, feasible, implementable plan for water users based upon site specific programmes, also taking into account the National Water Resource Strategy (NWRS), Catchment Management Strategy (CMS), Resource Quality Objectives (RQO's) and sensitivity of the receiving water resource, upstream and downstream cumulative impacts of water use activities, external water use authorisation guidelines, as well as water use specific supplementary information requirements. It is important to note that all Integrated Water Resource Management (IWRM) principles need to be considered as part of the IWWMP development process. The development of a site specific IWWMP is dependent on the risk categorisation of the activity. The risk related to a specific activity will be confirmed by the responsible regional office during the pre-application consultation with DWS. It is important to note that DWS and/or Catchment Management Agencies (CMA) implement IWRM at source by means of an IWWMP.

The National Toxicity Monitoring Programme

The NTMP was developed by DWS to be a "status and trends" monitoring program of water quality relating to toxicants and its toxicity (DWAF, 2005 and DWAF, 2006). Through the use of various aquatic ecological bioassays 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).

In addition 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 DWS 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.

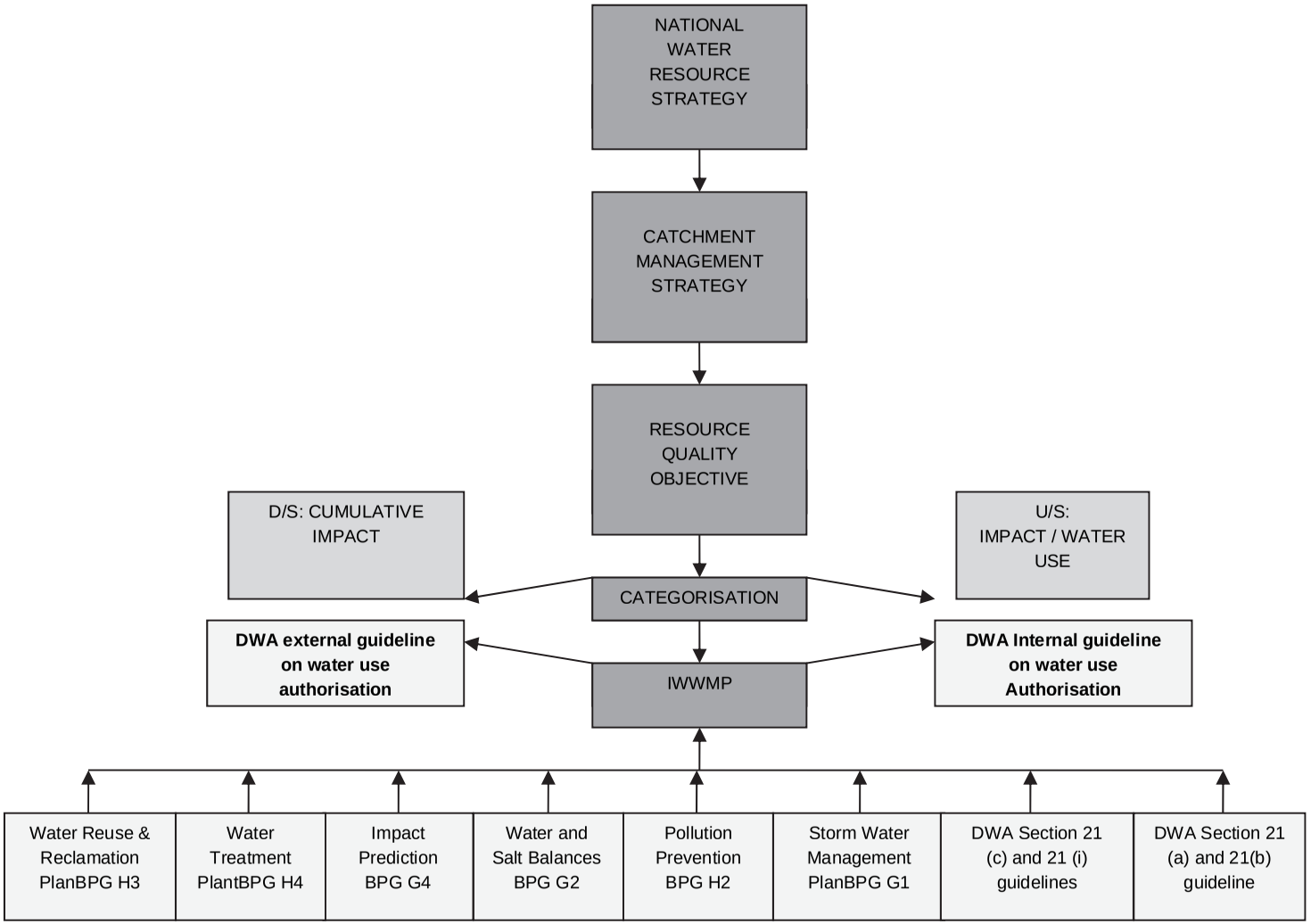

Figure 1-6: IWWMP approach (DWA, 2010)

Direct Estimation of the Ecological Effect Potential approach

The DEEEP approach for effluent/wastewater monitoring has been implemented since 2005 as a pilot study by DWS. The DEEEP approach assesses the ecological hazard posed by complex industrial wastewater on receiving ecosystems. 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 DEEEP approach was proposed as a means of circumventing the shortcomings of direct toxicant monitoring. DEEEP proposes a battery of tests to directly assess effluent oxygen demand, lethal (acute) and sub-lethal (chronic) toxicity, bioaccumulation, mutagenicity and persistence potential of effluents, using test organisms from a range of trophic levels. 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 and
- persistence.

Some of the anticipated key success factors in the implementation of the DEEEP approach were the following (DWAF, 2003):

- 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 South African National Water Act, 1998.

DEEEP is currently being phased into utilize the experience and skills available in the country, to test and refine the methods, and to allow for the creation of systems for training and skills transfer and for information management. The first step towards implementation of the DEEEP approach was the compilation of a "Methods Manual" addressing the selected ecological hazard parameters and tests to assess these parameters (Slabbert, 2004).

The assays proposed under DEEEP are all widely used and widely tested internationally. A criticism may be raised that these assays, relying on standard test organisms, may have little ecological relevance in South Africa (Griffin et al., 2011).

There is at present a Department of Water Affairs (DWA) funded project reviewing the derivation of South African Water Quality Guidelines, and consequently an opportunity exists to begin investigating the incorporation of episodic toxicity test data in managing environmental water quality. This project also provides an opportunity to assess the incorporation of episodic toxicity data in the direct toxicity assessment method proposed by DWA (Direct Estimation of Ecological Effect Potential (DEEEP) (DWAF, 2003).

In addition and not just limited to the DEEEP according to the WRC (2011) the following were identified to benefit from toxicity test information:

- Resource context – Classification and setting resource quality objectives;
- Reserve determination – basic human needs;
- Reserve determination – aquatic ecosystems;

- Monitoring ecosystem health;
- Monitoring compliance with resource quality objectives;
- National status and trends monitoring;
- Source context – Pollution prevention;
- Emergency incidents;
- Licence conditions.

1.2.7.2 Historic overview of the use of Ecological Bioassays in South Africa

Biological tests for water toxicity testing have been developed and evaluated by the Division of Water Technology (DWT) since the late 1970's in South Africa. These developments were initially aimed at establishing tests for the evaluation of drinking water quality. Since the late 1970's the emphasis was on the development of microbial toxicity tests, resulting in the establishment of several rapid toxicity tests. The acknowledgement of whole effluent bioassays as an appropriate tool to assess the suitability of hazardous effluents for discharge into receiving waters (DWAF, 1991) resulted in applied research to give effect to the provisions of chapter 3 of the South African National Water Act (RSA,1998). Prominent contribution from the Development of Procedures to Assess Whole Effluent Toxicity (Slabbert et al., 1998a) and Development of Guidelines for Toxicity Bio-assaying of Drinking and Environmental Waters in South Africa" (Slabbert et. al, 1998b) provided sound baseline data in this regard. These reports provide guidance on procedural issues as well as the suitability of locally available tests.

1.2.7.3 Collaborative agencies

Aquatox Forum

The Aquatox Forum was formed on 14 January 1998 by a group of scientists with a common interest in aquatic toxicity testing and legalized as a non-profit organisation on 11 February 2005. The vision of the Aquatox Forum was to serve as platform for the advancement of aquatic toxicity testing in South Africa and the focus area was the aquatic environment, drinking water and urban and industrial wastewater. Membership included people from Universities, science councils, industry, water boards, water treatment companies, supplier companies, contract laboratories, consultants and government.

The Forum objectives set out was:

- To pro-actively assist legislators and regulators to formulate and implement credible toxicity test requirements;
- To drive the standardisation of toxicity tests in collaboration with an acceptable standardization body;
- To establish a network for communication, liaison and information sharing between water toxicologists, regulating authorities, the public sector and industry (nationally and internationally);
- To assist in the promotion, publication and implementation of toxicity tests;
- To assist and advise in the training and education of interested and affected parties;
- To assist in the identification and formulation of viable and necessary research;
- To adopt and prescribe a code of conduct for its members that is in line with the best practice and professional ethics.

Water Research Commission (WRC)

The WRC is an internationally recognised scientific and research co-ordinating institution and is has over many decades been the centre of knowledge expansion and dissemination. Its role in the field of bioassay development and application is summed up in the table below. This not only illustrates the scope and quality of South African research and commitment to enhancing water quality, but also the capacity to maintain knowledge bases and innovative application of findings. The international *status quo* of bioassays has underlined the immense value of reputable scientific agencies to support legislators and executive authorities in the application, review and understanding of the diverse and intricate methodology.

Table 1-5: WRC sponsored bioassay research project reports over three decades (available on www.wrc.org.za).

Year	*WRC report title*	*Authors*	*WRC report number*
1984	Rapid detection of toxicity in water using the oxygen uptake rate of mammalian cells as sensor	Slabbert JL; Steyn PL; Bateman BW; Kfir R	317
1985	Toxicity and mutagenicity evaluation of water coagulated with Moringa oleifera seed preparations using fish, protozoan, bacterial, coliphage, enzyme and Ames Salmonella assays.	Grabow WOK; Slabbert JL; Morgan WSG; Jahn SA	350
1986	Evaluation of interactive toxic effects of chemicals in water using a Tetrahymena pyriformis toxicity screening test	Slabbert JL; Maree JP	389
1996	The use of Selenastrum capricornutum growth potential as a measure of toxicity of a few selected compounds.	van der Heever JA; Grobbelaar JU	928
1998	Development of Guidelines for Toxicity Bioassaying of Drinking and Environmental Waters in South Africa.	Slabbert J.L.; Oosthuizen J.; Venter E.A.; Hill E.; Du Preez M. and Pretorius P.J.	358/1/98
1998	Development of Procedures to Assess Whole Effluent Toxicity.	Slabbert J.L.; Oosthuizen J.; Venter E.A.; Hill E.; Du Preez M. and Pretorius P.J.	453/1/98
2000	Microtox™ and *Ceriodaphnia dubia* toxicity of BKME with powdered activated carbon treatment™.	Kennedy KJ; Graham B; Droste RL; Fernandes L; Narbaitz R	1234
2002	The use of Daphnia SSP. and indigenous river invertebrates in whole effluent toxicity testing in the Vaal catchment.	CG Palmer and WJ Muller	815/1/02
2003	Treatment of Landfill Leachate from Hazardous and Municipal Solid Waste.	Schoeman J.J.; Steyn A.; Slabbert J.L. and Venter E.A.	1167/1/03
2004	Methods for the Direct Estimation of the Ecological Effect Potential (DEEEP).	Slabbert J.L.	1313/01/04
2004	Biomarker assays for the Detection of Sub-lethal Toxicity in the Aquatic Environment: A Preliminary Investigation.	Slabbert J.L; Venter E.A.; Joubert A., Vorster A.; De Wet L.P.D.; Van Vuuren J.H.J.; Barnhoorn I. and Damelin L.H.	952/1/04
2007	Development and Application of a Prokaryotic Biosensor System for the Evaluation of Toxicity of Environmental Water Samples.	Pillay B and Pillay D.	1286/1/07
2010	Evaluating the contribution of episodic toxicity data to environmental water quality management in South Africa.	Gordon AK; Mantel SK; Muller WJ	KV259/10
2010	The Application of Chronic (Sub-lethal) Toxicity Endpoints for the Development of Resource Quality Objectives.	Gordon A.K.; Slaughter A.R. and Muller W.J..	1484 /1/09
2010	Development of Protocols for Acute Fish Toxicity Bioassays, Using Suitable Indigenous Freshwater Fish Species.	Rall V.E.; Engelbrecht J.S.; Musgrave H.; Rall L.J.; Williams D.B.G. and Simelane R.	1313/2/10
2011	Guideline for the Accreditation of Routine Aquatic Toxicity Testing	Chapman A.A.; Venter E.A.; and Pearson H.	TT 504/11

	Laboratories.		
2011	Aquatic Toxicity Testing in South Africa: Status of Aquatic Toxicity Testing in South Africa.	Chapman A.A.; Venter E.A. and Pearson H.	1853/1/11
2011	A Gap Analysis of Water Testing Laboratories in South Africa.	Balfour F.; Badenhorst H and Trollip D.	488/11
2011	Guidance for the Selection of Toxicity Tests in Support of the Information Requirements of the National Water Act.	Slabbert J.L. and Murray K.	1211/1/10
2011	Development of chronic toxicity test methods for selected indigenous riverine macro-invertebrates.	Muller WJ; Slaughter AR; Ketse N; Davies-Coleman HD; de Kock E; Palmer CG	1313/3/11
2011	Implementation of Ecological Hazard Assessment of Industrial Waste Discharge: A Comparison of Toxicity Test Methods.	Griffin N.J.; Muller W.J. and Gordon A.K.	KV 276/11
2012	The Identification of a Suitable Culture Organism to Establish a Bio-Assay for Evaluating Sediment Toxicity.	Cloete Y. and Shaddock B.	TT 532/12
2012	Rapid in vitro tests to determine the toxicity of raw wastewater and treated sewage effluents.	Hendricks R; Pool EJ	Short communication 2717
2013	Framework Document for a WRC Research Programme on Engineered Nanomaterials.	Wepener V.; Mamba B. and Musee N.	TT 549/12
Current projects	The selection and validation of sediment toxicity test methods to be included in the National Toxicity Monitoring program.	Shaddock B.F. Cloete, Singh, Motsumi	K5/2160/1
	Development of research support to enable the issuing of aquatic toxicity based water use licenses.	Pearson H. and Shaddock B.F.	Consultancy proposal
	Determination of the status quo of Persistent Organic Pollutants (POPs) and Emerging Pollutants research in the water value chain including upstream activities (return flows), catchment (water resources) and offtake to tap (drinking water).	Project not awarded yet	Consultancy proposal

In a WRC report published by Chapman et al., (2011a) it was 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.

Laboratories

As indicated in the above mentioned WRC report (Slabbert et. al, 1998b) it is evident in Table 1-6 that only four laboratories were actively performing aquatic toxicity tests in the late 1990’s.

Table 1-6: Locally available bioassays for freshwater testing according to Slabbert et al., (1998).

Laboratory	*Type of test*	*Bioassay*	*Test organism/ mammalian cells/enzyme specificity*	*Method*	*Exposure time*
Division of Water Technology (DWT), CSIR	Acute	Fish lethality test	*Poecilia reticulata* – guppy	EPA	96 h
		Daphnia lethality test	*Daphnia pulex*	EPA	48h
		Protozoan oxygen uptake assay	*Tetrahymena pyriformis*	Developed at DWT	10 min
		Algal growth Inhibition assay	*Selenastrum capricornutum*	Miniaturized assay based on EPA flask test developed at DWT	48-72 h
		Bacterial growth inhibition assay	*Pseudomonas putida*		6h
		Urease enzyme inhibition test	Heavy metals	Developed at DWT	15 min
		Acetylcholtnesterase enzyme inhibition test	Organophosphate and carbemate pesticides	Developed at DWT	15 min
		Mammalian cell colony formation inhibition test	BGM and V79 cells	Developed at DWT	6-8 days
	Chronic	Toad embryo teratogenicity test	*Xenopus laevis*	Developed at DWT	48 h
	Genotoxic	Ames mutagenicity test	*Salmonella typhimutium* (strains98and 100)	EPA	48 h
		Cell transformation assay	Hamster embryo cells	Developed at DWT	8 days
Rand Water	Acute	Fish lethality test	*Poecilia reticulata* – guppy	EPA	96 h
		Daphnia lethality test	*Daphnia pulex*	EPA	48 h
		Algal growth inhibition assay	*Selenastrum capricornutum*	EPA	72 h
		Bacterial growth inhibition assay	*Pseudomonas putida*	Developed at DWT	6h
	Genotoxic	Ames mutagenicity test	*Salmonella typhimurium* (strains 9S and 100)	EPA	48 h
Institute for Water Quality Studies – IWQS	Acute	Fish lethality test	*Poecilia reticulata* – guppy	EPA	96 h
		Daphnia/Ceriodaphnia lethality test	*Daphnia pulex / Ceriodaphnia dubia*	EPA	24/48 h
		Algal growth inhibition assay	*Selenastrum capricornutum*	EPA	72 h
		Bacterial growth inhibition assay	*Pseudomonas putida*	Developed at DWT	6h
	Chronic	Invertebrate reproduction test	*Daphnia pulex/ Ceriodaphnia dubia*	EPA	7-21 days
Catchment and Coastal Environmental Program, CSIR	Acute	*Daphnia* lethality test	*Daphnia pulex*	EPA	48 h
		Algal growth inhibition assay	*Selenastrum capricornutum*	EPA	

In a subsequent 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. The closure of the Council for Scientific and Industrial Research's (CSIR) Pretoria aquatic toxicity testing laboratory in 2008 left a significant research and development gap in the field of toxicity testing.

In 2011 the number of Aquatic Toxicity Laboratories increased to 28 (Figure 1-7) (Chapman et al., 2011a). According to this report it was recommended that toxicity testing facilities operate within regional laboratory networks supported by DWS and the South African National Accreditation System (SANAS).

Figure 1-7: Locality of aquatic toxicity testing facilities and individuals involved with aquatic toxicity testing in South Africa, June 2009 according to Chapman et al., (2011a).

This is a clear indication that independent quality assurance provisions can be met as illustrated by the growth in service providers and those seeking accreditation in terms of regulatory requirements. It was recommended that:

- Networks between laboratories that have already achieved accreditation for the purpose of toxicity testing and those laboratories seeking accreditation status be set up to foster a culture of cooperation to achieve national objectives;
- Capacity in terms of testing facilities and human resources be increased. Existing facilities need a regulatory incentive from government to maintain existing capacity.
- Affordable human capacity building 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.

As a result of these findings a Practical course on Aquatic Toxicity Testing was initiated by the Aquatox Forum in collaboration with the National Laboratory Association NLA in 2013. The aim of course was to introduce aquatic toxicity tests as acceptable tools for the management of complex industrial wastewater discharges in accordance with the “Direct Estimation of Ecological Effect Potential (DEEEP)" approach by DWA, 2003. The course overview includes:

- Introduction to the “Direct Estimation of Ecological Effect Potential (DEEEP) approach by DWA, 2003.
- Quality Control and Quality Assurance practices.

- Sampling and preparation of samples.
- Performance of physical and chemical parameters (including calibration, maintenance and verification of equipment).
- *Vibrio fischeri* bioluminescent test.
- *Selenastrum capricornutum* growth inhibition test.
- *Daphnia* acute toxicity test.
- *Poecilia reticulate* acute toxicity test.
- Documentation of results and information obtained during analysis.
- Verification procedures including control charts and Proficiency Testing results.
- Reporting and discussion of results.
- Interpretation of results such as the use of Hazardous Classification Systems

SABS

In 2011 the sub-committee SC147B responsible for Water, Microbiological and Biological Treatment of Water agreed to include the development of national standards in the field of aqua toxicity testing in their work programme. The request emanated from existing toxicological test labs and the focus is on the review and updating ISO standards as well as adopting ISO methods.

Table 1-7: List of SABS adopted aquatic toxicity standards.

ISO 6341:2012*	Water quality -- Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) -- Acute toxicity test
ISO 7346-1:1996#	*Water quality -- Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] -- Part 1: Static method*
ISO 14371:2012#	Water quality -- Determination of fresh water sediment toxicity *to Heterocypris incongruens* (Crustacea, Ostracoda)
ISO 8692:2012*	*Water quality -- Fresh water algal growth inhibition test with unicellular green algae*
ISO 11348-3:2007#	*Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria*
ISO 14380:2011#	*Water quality -- Determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca)*
ISO 20665:2008#	*Water quality -- Determination of chronic toxicity to Ceriodaphnia dubia*

\# *Standards adopted by the South African Bureau of Standards (SABS).*

* *Standards in the process of adoption by the South African Bureau of Standards (SABS).*

1.2.8 METHODOLOGY

1.2.8.1 Definition

Within the South African context a toxicity test method is defined as an experimental procedure that measures, under defined conditions in the laboratory or in the field, the toxic effects of chemical pollutants in water on a group of living organisms, or a cellular, or a sub-cellular system." (Slabbert et al., 1998b). In essence the above mentioned definition excludes measurement of the pollutants themselves, bioaccumulation, biodegradation, and direct effects of turbidity, pH and temperature. Reference, however, must be made of the vital importance of research and policy formulation relating to the identification and status of compounds emanating from industrial, agricultural and pharmaceutical processes. Of particular concern is the cumulative effect of these substances in biota on all trophic levels. This is substantiated by Dallas and Day (2004) and Kleynhans and Louw (2008) in their comprehensive reports on the effects of variables on aquatic ecosystems.

1.2.8.2 Quality Assurance

The reliability of analytical data goes hand in hand with the quality of such data, which, in turn, depends on the quality assurance (QA) and quality control (QC) practices applied by the test laboratory. According to the technical support document for toxics control (Slabbert et al., 1998a), precision and accuracy in the execution of the toxicity test affects variability of the test results. Quality systems must therefore be put in place to limit the variations that can be caused by, e.g. differences in individual test organisms, test conditions and laboratory personnel competence (Chapman et al., 2011a).

Toxicity tests used for regulatory compliance must provide the same results when applied for the same chemical in different laboratories as well as for tests performed in the same laboratories at different times of the year. If the South African Direct Estimation of Ecological Effect Potential (DEEEP) approach is to be given legal standing to control and monitor point-source pollution in terms of licensing and setting licensing conditions, the toxicity tests which they are based on have to be legally justifiable. The scientific integrity of the method should therefore be indisputable: the method should be accepted as a “standard method” and the applicability of the method to provide the necessary information pertaining to the specific situation should be approved.

To deal with this ever increasing need for technical assistance with accreditation a WRC funded a project providing guidelines in this regard (Chapman et al., 2011a). Up to date requirements for accredited laboratories can be found at www.sanas.co.za.

This document provides an overview of the procedure for applying for accreditation in South Africa. It also describes a four-tier quality management system and related documents. The accompanying DVD contains these latter documents (about 200), each in a separate file for convenience. They are in MS Word format to allow managers of toxicity laboratories to customise them to their requirements. Hyperlinks have been installed to access relevant information in other sections, e.g. a standard operating procedure or a blank form, etc., and also to access information on the Internet. The document highlighted the fact that accreditation is not commercially viable for the smaller facilities that undertake toxicity tests.

As indicated in Table 1-8, three laboratories are currently accredited for aquatic toxicity tests and according to the authors five more laboratories are in the process to obtain accreditation status for similar methods.

Table 1-8: ISO 17025 Accredited Toxicity Testing Laboratories in South Africa.

Laboratory name	***Laboratory number***
Umgeni Water in Pietermaritzburg	T0036
Rand Water Analytical Services in Vereeniging	T0046
Golder Associates Research Laboratory in Johannesburg	T0384
Resource Quality Services (DWA) in Pretoria	In the process
BioTox Laboratory	In the process
Vaal University of Technology	In the process
eThekwini Local Municipality	In the process
CSIR, Durban	In the process

One of the recommendations of a WRC report by Chapman et al., 2011b suggested the establishment of a National Toxicity Testing Laboratory Accreditation Programme. This process has been started through the implementation of a PTS scheme by the NLA during 2014.

1.2.8.3 Species Selection

Muller et al. (2011) emphasised the importance and value of chronic toxicity assessments and the long term effects of toxicity. This WRC report entitled “Development of Chronic Toxicity Test Methods for Selected Indigenous Riverine Macro Invertebrates” contains valuable data relating to the decline in the diversity of aquatic biota by the cumulative effect of pollutants and the use indigenous species as *Caridina nilotica* as test organisms. The use of chronic assessments should be taken account when water use licences are considered and would enhance the objectives of those agencies and scientists involved in the maintenance of aquatic systems.

Note must also be taken of the suggested use of biochemical markers as sub-lethal endpoints in water resource management although it does not fall within the scope of the present project (Gordon et al., 2011).

The use of indigenous species is universally debated and researched. Advantages such as being more suitable under local conditions and availability are constantly weighed up against international application and duplicity. One such project (Rall et al, 2010) provided valuable information on *Caridina nilotica* which was deemed a suitable species for local acute toxicity testing. The project objectives included:

- To develop capacity to ensure the continued production of adequate test organisms for research and consulting facilities in South Africa in order to meet market requirements;
- To establish protocols for the laboratory culturing and maintenance of selected indigenous freshwater fish species, for use in eco-toxicity testing;
- To establish fish bioassay protocols which will provide representative data for ecosystems in the South African context.

1.2.8.4 Selection of ecological bioassays

A step towards implementing the DEEEP approach was to establish a guide for the selection of toxicity tests that support the information requirements of the South African National Water Act (RSA, 1998). Slabbert and Murray (2011) proposed a guide for the selection of toxicity tests to support the information requirements of the National Water Act. The authors acknowledge that terminology may sometimes be a hindrance in the communication process between scientists and managers/legislators. The term ‘toxicity test’ as an example, often has different meanings for scientists and water quality managers and to ensure a common understanding (Table 1-9).

Table 1-9: Water quality management toxicity test criteria (Slabbert and Murray, 2011)

Criterion			Description
Generic management criteria	Screening / Definitive		Whether or not a toxicity test is applied directly to a test sample, without sample dilution, as in the case or a resource water (screening), or if dilutions of the test sample requires testing, as in the case of an industrial effluent (definitive).
	Legally defensible		A toxicity test is regarded as legally defensible if one or more of the following requirements are satisfied with regard to the experimental method and the reliability of the experimental result: • The test has been successfully defended in a court in the past • It is a "standard test" (i.e. appears in ISO, US EPA, Canadian, South African, etc. documentation) • It has been published substantially in scientific literature and • It has been shown to be reproducible (reliable) in inter-laboratory studies.
	Effect period		The effect period can be short-term (acute) or long-term (chronic). Short-term refers to a short period of time (hours to a few days) in relation to the life span of the test organism / material while long-term refers to a long period (weeks or more).
	Target kingdom		Two target kingdoms were identified, namely the plant kingdom and the animal kingdom. The decision on which target kingdom a toxicity test represents assumes the more lenient "protective approach", rather than the "predictive approach".
	Max days turnaround		The time it takes from the sample receipt in the laboratory until the test report is completed.
Other management criteria	Target Kingdom		The description is the same as the generic management criterion "target kingdom" above
	Optional criteria	Specificity	Whether or not a toxicity test is selectively sensitive to a specific group of chemicals e.g. heavy metals, pesticides, etc.
		Interferences	Whether or not certain properties of a water, a discharge or an extract, e.g. colour, salt content, etc. can interfere with a toxicity test.
Generic types of water sources	Applicability	Water resource type	Whether or not a toxicity test can be applied to an inland water resource (e.g. watercourses, impoundments, wetlands, etc.) or an estuary.
		Water resource zone	Whether or not a toxicity test can be applied to the water body, sediment or groundwater.
		Water resource, discharge or extract water quality	Whether or not the toxicity test can be applied to fresh (low salinity) or brackish (high salinity) water.
		Solid or non-aqueous liquid	Whether or not the toxicity test can be applied to an extract or a leachate of a solid waste (chemical or domestic) or a stockpile or a chemical solution (prepared with water or an organic solvent)

More than 100 toxicity tests used locally and internationally were analysed and their value and application defined in context of South African National Water Act (Act 36 of 1998) objectives and management criteria. The resultant spreadsheet facilitates the choice of appropriate tests for resource directed measures (i.e. Monitoring and trends) as well as and source directed controls (i.e. licensing). It was suggested that the maintenance of these guidelines resides in the WRC which raises the question on the procedures regarding the updating and maintenance of standards and procedures. The authors indicate that because of the limited understanding of the application potential of toxicity tests, most of the local tests have been applied in hazard assessments to establish toxicity at the source level. The situation in South Africa, however, requires us to have methodologies available for resource directed measures and source-directed controls, and to understand how methodologies for one application relate to the other. It has become necessary to

contextualise those tests already available in South Africa and elsewhere, and to identify the gaps with reference to specific tests, so that we can satisfy the information requirements of the NWA. The objective of the project was to establish a guide for the selection of toxicity tests that support the information requirements of the South African National Water Act (Act 36 of 1998) and of particular interest is the table which summarises the tests and their application.

1.2.8.5 Standards, limits, procedures and sampling

The design and ultimate testing of the proposed Integrated Water Use Authorisation Bioassay Toolkit (Chapter 3) is to be done in collaboration with DWS and all affected parties and this process has been initiated. Decisions and consensus on technical issues will be based on international and local knowledge bases and experience collated in this report, and the needs of affected role players. The integrated and multi-disciplinary approach to water quality has to be kept in mind in spite of the fact that the main focus of the proposed project is the design of an instrument to facilitate water use licenses. In this regard the following factors are important:

- Selection of toxicity tests should reflect the nature and scope of individual water users. Uniform prescriptions for generic components such as mining for instance, is not possible as each deliver different potentially hazardous substances to receiving waters and the chemical composition and resident biota can have an influence on test methods;
- Current and contemplated policy guidelines may affect frequency and selection of sampling sites i.e. above, below and downstream of effluent release;
- A collaborative selection of specific procedures and tests by the various role players may be mutually beneficial for example to enhance monitoring projects and predictive capabilities;
- As for above it can provide extensive and reliable data bases which will facilitate the attainment of protection/enforcement and environmental objectives

1.3 LITERATURE REVIEW SUMMARY

The broad review of the local and international *status quo* of aquatic bio-assays or toxicity testing clearly indicates that all requirements for formal implementation of this most valuable instrument to enhance quality water in South Africa can be met. The following observations from the data may provide valuable guidance in the process of legislation/policy development and practical implementation of these bio-assays in water management, in particular the issue of water use licences:

Global status

Aquatic bio-assays are globally recognised and applied as integral component of water resource- and source management practices. Formal (legislative) application is in many cases hampered by factors such as cost implications, capacity related issues and in some instances ignorance of the value of the instrument by decision makers. The lack of formal recognition can also be correlated with the development stage of states, and to a lesser extent paucity or abundance of water resources. The global importance of these bio-assays is illustrated by the exponential growth in research and technology in this field over the last three decades.

Multiple applications

Bio-assays are cost effective instruments and can be used for diverse purposes. Though mainly known and applied for compliance and routine monitoring, it can be used to evaluate the scope and effect of catastrophic incidences on biota, to determine long term effect of lethal and sub-lethal substances and for sophisticated screening purposes. Although not within the scope of this study it is thus evident that the instrument potentially has a broader application by government and non-government agencies responsible for human and environmental welfare.

Interstate co-ordination

The most successful models to ensure a co-ordinated, uniform and responsive approach to bio-assays are found in formal inter-state agreements which provide for guiding principles, advanced technology and procedures. Most prominent examples include those for the European Union, Australia and New Zealand, and though not strictly “inter-state”, the United States of America. This concept enhances dissemination of knowledge, technology and experience and ensures uniformity and coherence.

It must be noted that most of the South African neighbouring states have limited application of toxicity testing in their water management strategies and it is imperative that they be exposed to the value and application thereof. Many have limited water resources and also share responsibility for this strategic resource due to cross border aquatic systems and climate zones. South Africa has the capacity to share knowledge and provide guidance through forums such as SADEC and other interstate agreements.

Enabling legislation and policy

The South African National Water Act (Act 36 of 1998) enables the implementation of a wide scope of measures to facilitate the innovative and practical water management instruments and procedures. This includes formal prescriptions by way of regulations or formal directives which would facilitate regional or local initiatives. As a strategic resource, water will play a crucial role in the present development initiatives of government. It is of vital importance that sound and practical policy decisions recognise the needs and rights of regional and local communities.

Delegation and assignment of authority

Most countries favour a central policy making and regional (local) implementation model. This enhances grass roots participation and acceptance of water related issues and more efficient resolving of problems at local level. The establishment of proposed Catchment Management Agencies (CMA) and with assigned authority is thus an essential component of the decision making process. The current procedure for the issue of Water Use Licences (WUL) illustrates the value of a more decentralised system.

Inter departmental involvement and co-operation

There is at present substantial legislation (central, provincial and local) aimed at maintaining the viability versatility of renewable and non-renewable natural resources. The more prominent departments and agencies include those responsible for the environment, agricultural-, industrial- and development related legislation. In most developed countries formal relationships between these role players provide the basis for co-operation in matters such as monitoring and compliance. This alleviates the burden of the primary water management authority. The sharing of expertise and experience, data bases and mutually beneficial policy and legislation are obvious advantages.

Role of non-government agencies

The role and value of non-government agencies is emphasised in most countries. For obvious reasons research and development agencies play a prominent role but it is the formal relationship between these agencies and decision makers which will ultimately ensure the optimal application of innovative technology. This is also applicable to interest groups. In this regard the Water Research Commission has played a prominent role as research initiator and co-ordinator for many decades as can be seen from the South African data presented. Independent quality control is of vital importance and laboratories and test centres have to cater for the volume of prescribe tests and evaluations. The indications are that the local component will be able to handle the require volume and standard of tests. Accreditation of these laboratories and test centres will be necessary. As in the case of interdepartmental liaison and co-operation the relationship should be formalised

Classification/registering of water users

Many governments require the mandatory registering of water users, and more specifically manufacturers and processors of potentially harmful substances, industrial and other plants which use/release water which

physically and chemically affect the aquatic environment and agricultural usage where chemical and other substances affect water systems. In a South African context the classification of users would be of significant importance and would facilitate the monitoring, enforcement and licensing function of government.

Consultative process

The technical data condensed in the desk study and the observations above are aimed at providing guidance for formulation of a technical guideline which will precede a comprehensive pilot study. The guideline will include the selection and standardisation of tests, sampling procedures frequencies, limits and training required for implementation in the issue of water use licences and compliance related policy. Discussions have been held with officials from DWS and non-government agencies and valuable comments have been received.

CHAPTER 2: TOXICITY ASSESSMENT APPROACHES

2.1 INTRODUCTION

Internationally the ability to detect acute and chronic toxicity plays an increasing role in identifying and controlling the toxicity of effluent/wastewater discharges to receiving/surface water. Widespread experience in effluent testing has shown that even discharges, which had passed chemical quality criteria imposed by competent authorities, could be acutely toxic to aquatic life (Heber et al., 1996). In other words, effluent limitations on specific wastewater constituents do not necessarily provide adequate protection for aquatic life. Additionally, in many of these cases the actual toxicity of the wastewater constituents is not known. In contrast assessing effluents with bioassays enables the detection of additive, synergistic, or antagonistic effects (US EPA, 1995). In the case of positive results, detailed chemical analysis should be carried out.

The objective for this guideline document is to layout the theory and procedures associated with the protection of aquatic ecosystems. The information available has been combined in order to produce a concise reference with information on the background and procedures recommended to measure the toxicity of complex effluents/wastewater released into the environment. The correct selection of relevant ecological bioassays is necessary to maintain and enhance the 'ecological integrity' of these freshwater ecosystems, including biological diversity, relative abundance and ecological processes.

As in South Africa, the first testing guidelines were developed by the US EPA in the early 1970s. In an effort to obtain data on chronic effects of effluents in a cost-effective manner, the US EPA began developing short-term toxicity tests for estimating chronic toxicity in 1980 (freshwater 4-7d; saltwater 1h-9d). Since then, the number of ecotoxicology tests and the experience in performing tests has grown rapidly internationally.

Aquatic ecosystems consist of the animals, plants and micro-organisms that live in water, and the physical and chemical environment and climatic regime in which they interact. It is predominantly the physical components (e.g. light, temperature, mixing, flow, and habitat) and chemical components (e.g. organic and inorganic carbon, oxygen, nutrients) of an ecosystem that determines what can live and breeds in it, thereby affecting the structure of the food web. Biological interactions (e.g. grazing and predation) can also play a part in structuring many aquatic ecosystems.

"Whole Effluent Assessment" (WEA), including chemical and ecotoxicological characterisation of effluents should be applied on a case by case basis, and used as guidance for issuing water use licences. Such assessments are normally performed on grab or composite samples of the final effluent as well as samples collected from sites up and down stream of the final effluent release site. The chemical analysis programme should include common general water quality and summary parameters as well as specific analysis of selected pollutants, whilst the ecotoxicological evaluation should include bioassays using indicator organisms relevant to the site and industry.

Depending on whether the receiving ecosystem is non-degraded or has a history of degradation the management focus can vary from simple maintenance of present effluent quality to improving current effluent quality so that the condition of the ecosystem is more natural and that the ecological integrity is enhanced.

Literature studies (OSPAR, 2000) agree that using site specific ecological bioassays to evaluate complex effluents have the potential to be an efficient additional tool for compliance monitoring, complementing physical-chemical and biomonitoring results, for example:

- to identify and characterise individual effluents;
- to identify industrial sectors which discharge these effluents;
- to develop targets/benchmarks for effluent quality and/or quality and receiving waters and
- to use this tool in the evaluation and development of Best Available Technology (BAT) for treatment.

Licenses must therefore state that the ecological bioassays undertaken are internationally standardised or validated methods performed by an accredited testing laboratory or reputable facility.

When characterising an effluent or executing compliance monitoring, the licensee is required to undertake an initial screening test with test organisms from a minimum of three different trophic levels. These three trophic levels can be broadly categorised as any of the following: bacteria, algae, plants, invertebrates and vertebrates. In addition to the requirement for toxicity monitoring, the licensee may also have to comply with a toxicity limit expressed in Toxic Units (TU) which also takes into account the dilution available in the receiving system.

2.2 CONCEPT OF TOXICITY

According to Slabbert et al. (1998a) “toxicity” is defined as the characteristic of a chemical (or a group of chemicals) that causes adverse effects in organisms. Toxicity is measured by observing the responses of organisms to increasing concentrations of a chemical substance. A substance can be classified as more toxic than another when the same adverse effect is caused at a lower concentration. Generally, for any given substance, toxic effects can be alleviated when concentrations are reduced. Thus, when the toxicity of a discharge is reduced (concentrations of toxic constituents reduced), the toxic effect of that discharge on receiving waters is also reduced. Similarly, greater dilution of a toxic discharge will result in lower toxicity in receiving waters (US EPA, 1990). Adverse effects can include mortality or those effects limiting an organism's ability to survive in nature, and can be acute or chronic (US EPA, 1990; 1991).

These can be defined as follows:

- **Acute** means a stimulus severe enough to rapidly induce an effect (short-term effects). In aquatic toxicity tests an effect observed within 96 hours or less is usually considered acute. An acute effect is usually but not always measured in terms of lethality/mortality.
- **Chronic** means a stimulus that continues for a relatively long period of time (long-term effects of small doses and their cumulative effects overtime). Chronic toxicity is measured in terms of sub-lethal effects such as reduced growth, reduced reproduction and so on in addition to lethality. Traditionally chronic tests are full-cycle tests or a shortened test of approximately 30 days and known as an early life stage test. Most of the US EPA's tests have been shortened to 7 days, and called short-term chronic tests.

2.2.1 LIMITATIONS OF A CHEMICAL APPROACH

Currently the physical-chemical specific approach plays a major role in compliance monitoring and water quality policy internationally. However, when considering complex mixtures such as effluents, the possibilities for a physical-chemical specific assessment are limited since:

- there are many substances which cannot be identified;
- not all substances can be analysed for or are detectable;
- the number of substances present can be so large, that a chemical specific approach is not feasible;

- there is a lack of data on effect-parameters for many substances;
- data on the environmental characteristics are not available or incomplete;
- micro pollutants and degradation products are undefined and therefore not accounted for;
- combined effects of substances, present in the discharges, are not being taken into account and
- the environmental characteristics of a mixture can differ significantly from those of single substances; (e.g. Tonkes et al., 1997).

2.2.2 REQUIREMENTS

For monitoring wastewater discharges, attention has focused on bioassays that meet the following requirements:

- legally defensible;
- internationally accepted standard with clearly defined endpoints;
- precise and reproducible methods;
- comparability of the results;
- sensitivity towards a large number of chemicals;
- measurement of biologically relevant toxic effects using organisms representative of the aquatic environment (juridical reliability);
- able to show clearly the success of wastewater treatment;
- practicable for routine measurements (test organisms available throughout the year, suitable for laboratory cultivation);
- moderately time consuming and having moderate equipment costs and able to rapidly provide
- unambiguous test results,
- maximum turnaround time (days),
- effect manifestation period (classification – short term or long term),
- target kingdom to be 'protected' (classification – animal or plant) and optional criteria relating to specific chemical groups (e.g. heavy metals, pesticides, etc.) present in water (specificity) (classification – yes or no);
- sample properties (e.g. very dark colour, etc.) that can interfere with toxicity tests (interferences) (classification – yes or no).

2.2.3 COST

The availability of resources is recognised as a major constraint in meeting the level of compliance monitoring. Ways to defray costs must always be considered. Some examples to consider in this respect include:

- As far as possible, ensure that there is a common sampling program for collection of data on different indicators. Other than providing greater interpretative value for the data gathered, this will reduce logistical costs (e.g. transport, etc.).
- Sharing testing costs with similar monitoring programs being conducted in adjacent areas.
- Incorporation of biological assessment in environmental monitoring programs may lead to cost-savings for industry if 'no-observable-effects' in biological responses are found, despite values for physical-chemical indicators that might be 'high' or which may exceed the recommended guidelines.
- In order to save costs and prioritise sites, screening tests (conducted on undiluted samples) should be conducted first. This will identify sites with high toxicity which would require additional testing using the definitive approach (geometric dilution range: 100%, 50%, 25%, 12.5%, 6.25%) and determination of LC/EC_{50} data.

2.3 MANAGEMENT STRATEGIES AND APPROACHES

2.3.1 BACKGROUND

The elements of ecological bioassays in management strategies can be incorporated into comprehensive planning practices such as integrated or total catchment management plans (ICM or TCM) or can remain relatively small-scale plans for local areas and industries. However, there must be consultation with stakeholders for the effective use and integration of a multi-disciplinary array of skills and knowledge to achieve success.

The management strategy and responses will depend on the issue of concern, the cause(s) of the poor water quality and the available tools, and should be negotiated and agreed upon by the local or regional stakeholders. Strategic management strategies can be in the form of catchment management plans or governmental policies such as the National Water Act (Act 36 of 1998) or the Direct Estimation of the Ecological Effect Potential (DEEEP) approach. These various management strategies should be based on agreed environmental values and their associated resource quality objectives. Regulation could be achieved through discharge consents and codes of practice designed to ensure water quality objectives are not exceeded and taking into account cumulative impacts from all sources.

After all available and technical information has been collated for a defined water body; a management framework (Figure 2-1) can be followed to implement a broad national management strategy at a local level.

- Identify the environmental values that are to be protected in particular the water body and the spatial designation of the environmental values (i.e. decide what values will apply where).
- Identify management goals and then select the relevant water quality guidelines for measuring performance. Based on these guidelines, set water quality objectives that must be met to maintain the environmental values.
- Develop statistical performance criteria to evaluate the results of the monitoring programs (e.g. statistical decision criteria for determining whether the water quality objectives have been exceeded or not).
- Develop tactical monitoring programs focusing on the water quality objectives.
- Initiate appropriate management responses to attain (or maintain if already achieved) the water quality objectives.

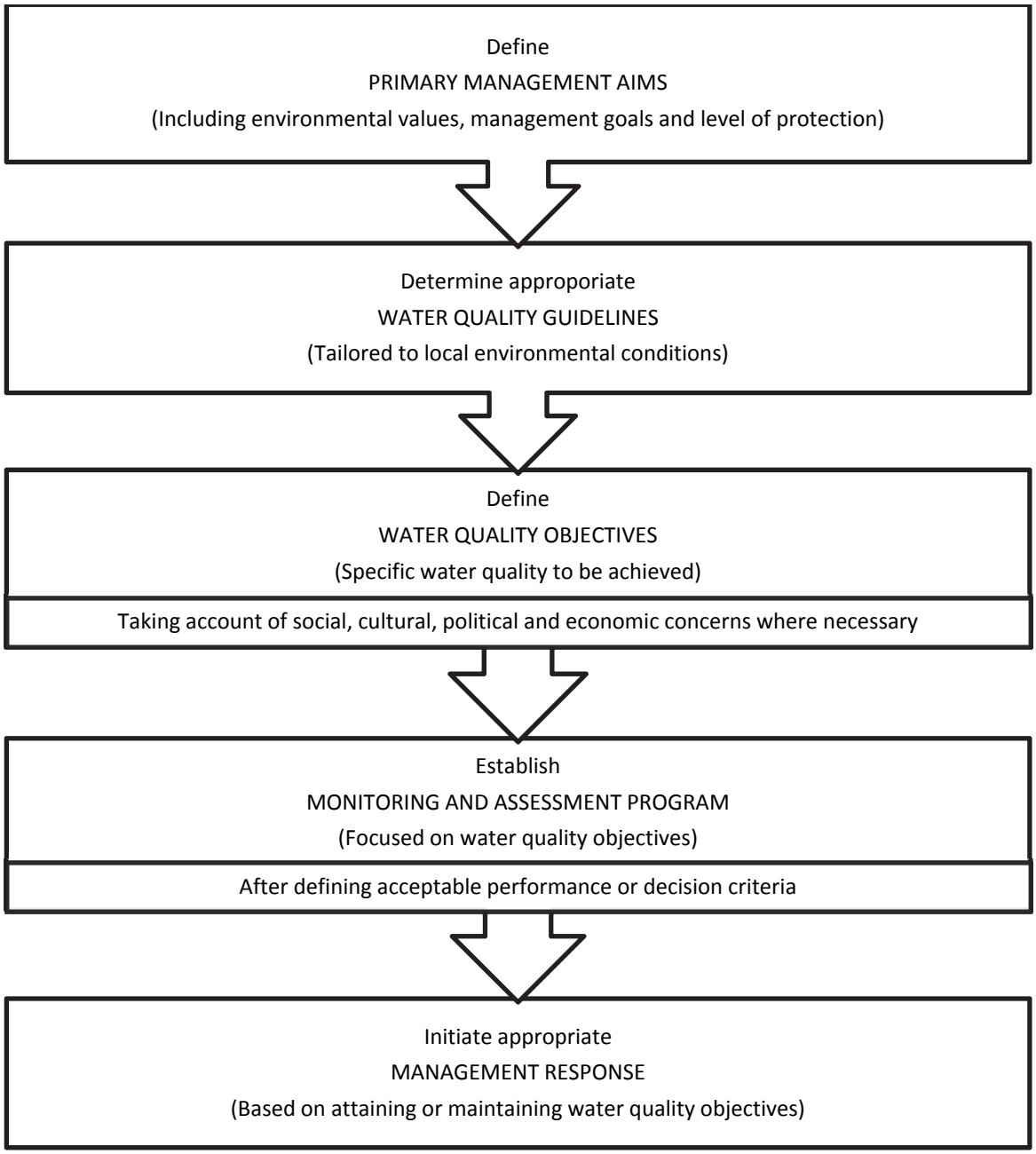

Figure 2-1: Management framework example (ANZECC, 2000).

2.3.2 DIRECT ESTIMATION OF THE ECOLOGICAL EFFECT POTENTIAL (DEEEP)

There are many instances where the potential ecological effect is not apparent from data obtained through chemical analysis for specific substances. Therefore, additional methodologies that are able to assess the potential impacts resulting from the whole wastewater discharge are required to provide an integrated assessment of potential effects (or hazard) of waste discharge.

The intention is not to replace substance-specific assessments, but to supplement them with assessments that can directly measure the potential effect of complex mixtures when the chemical composition of a discharge is not known. **Figure 2-2** illustrates how an integrated discharge hazard assessment can be achieved using a combination of the substance-specific (indirect) and effect-based (direct) assessment approaches.

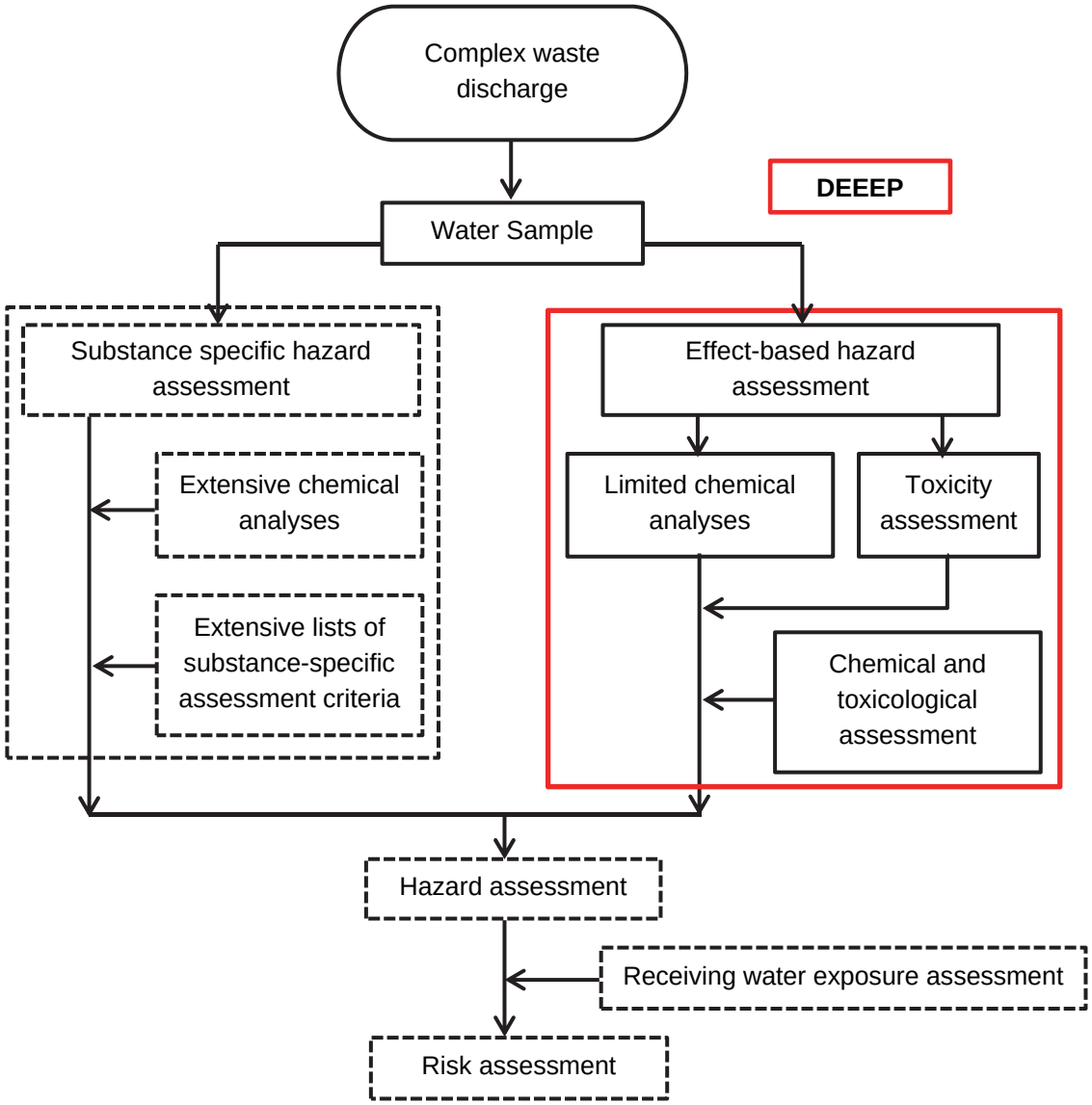

Figure 2-2: Approach example for discharge hazard assessment using a combination of the substance-specific and complex discharge hazard assessment approaches (the blocks indicated by dotted lines are not included in the DEEEP approach).

Such methodology was found to be particularly promising. The TEM methodology ("Totale Effluent Milieu hygiene" or "Whole Effluent Environmental Risk") was developed in the Netherlands. Its development took place in the nineteen nineties by RIZA, the Dutch Institute for Inland Water and Wastewater Treatment. The parameters and tests it uses to assess the hazard parameters are now well known internationally and well established in the scientific community. TEM includes a combination of parameters and tests and represents a suite of methodologies. This approach yields the kind of ecological hazard assessment that is required in South Africa, because:

- The TEM approach is sufficiently flexible to be adapted to local circumstances and to available capacity for conducting the necessary tests and applying the required parameters. Therefore, the proposal is to use TEM as a foundation for a South African direct hazard assessment, to be known as Direct Estimation of Ecological Effect Potential (DEEEP).
- The DEEEP methodology is conceptually well thought out and developed, and represents the culmination of 30 years of such development in the Netherlands, USA and UK. It is well tested and has been shown that it can be practically implemented. Hence, the Department believes it merits further investigation as a useful additional tool in the management and control of complex waste discharges.

- DEEEP's main attraction is that it provides a fairly direct ecological hazard assessment of known and unknown mixtures of substances. It can assess the ecological (and maybe even the human health) hazard of discharges within a coherent system of hazard parameters. Thus, it can provide the second of two legs in integrated hazard assessment (see Figure 1).
- DEEEP is equally useful in the assessment of complex industrial discharges, treated sewage point discharges and localised diffuse sources. It can be used by the regulator and the discharger alike to demonstrate environmental care.

The Department of Water Affairs and Forestry, as public trustee of the nation's water resources, has the mandatory function to protect water resources. Consequently, any suitable measure that will highlight ecological hazard of complex industrial discharges could be used in fulfilling this mandatory function. DEEEP would clearly fall in this category. As an assessment methodology, DEEEP is particularly attractive to the Department, since it is able to provide an up-front indication of the potential hazard of the discharge.

Due to the suite of parameters and tests from which to choose, the DEEEP (Figure 2.3) methodology can be practically implemented in a step-wise fashion over time.

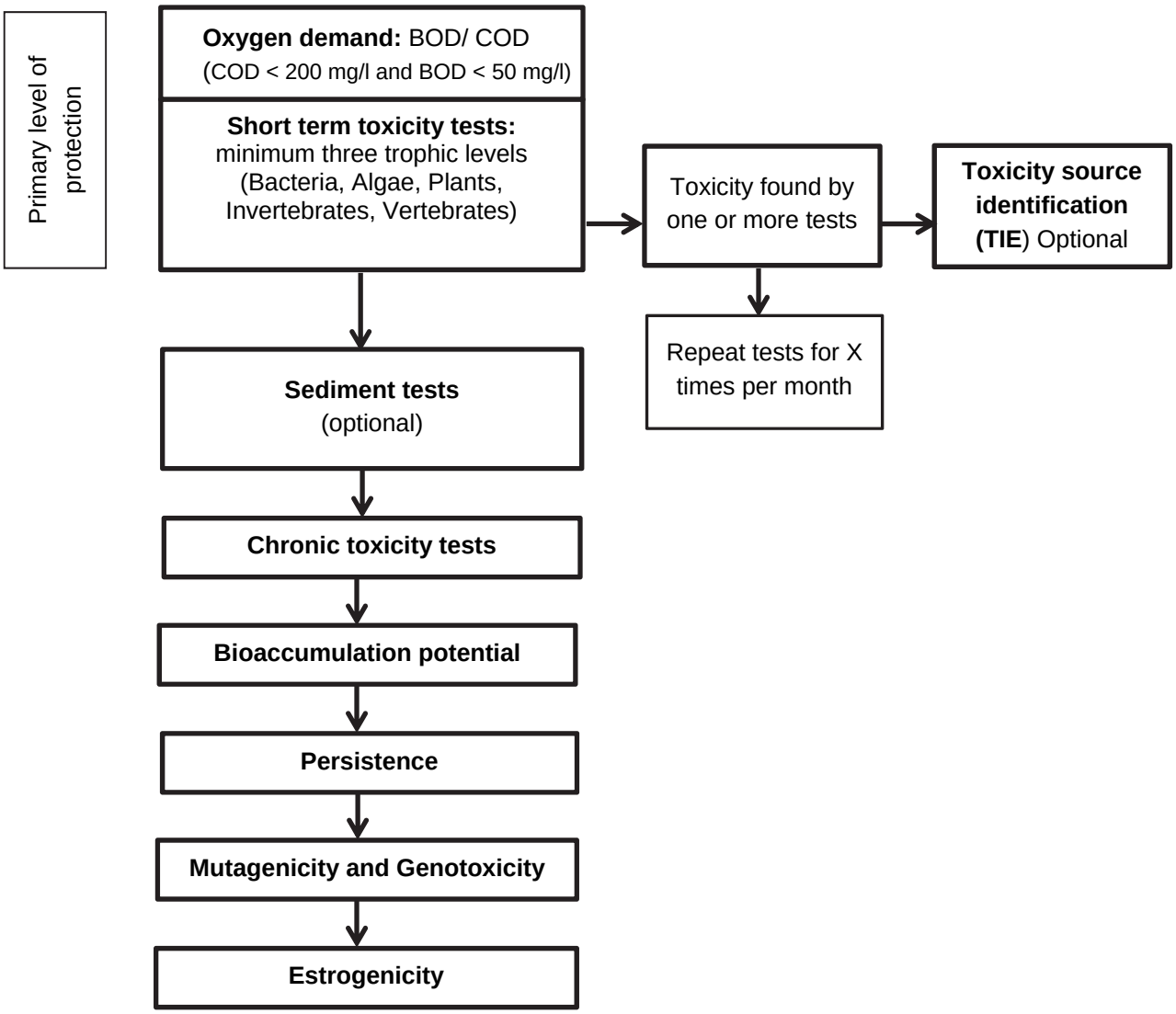

Figure 2-3: Updated Proposed DEEEP Approach

It should be noted that the assessment of truly diffuse sources (such as polluted groundwater percolating into a stream) will not benefit from DEEEP any more than from a substance-specific approach. However,

where spatially localised sources of hazardous substances are found (such as a landfill site) DEEEP will provide a better overall view of the environmental hazard than a substance-specific approach.

2.3.3 EXISTING FRAMEWORKS

2.3.3.1 Integrated Wastewater Management Programmes (IWWMP)

The incorporation of the aquatic toxicity tests into existing frameworks would be done with minimal disruption. The existing process of compiling Integrated Wastewater Management Programmes (IWWMP) already highlights areas of application, which are **highlighted** below.

The following process is followed with IWWMP (DWA, 2010):

Step 1
During the *Pre-application consultation stage* of the Water Use Authorisation Process the water user needs to consult with DWS in a meeting to discuss the relevant strategies applicable to the specific activity, namely the **NWRS, CMS, WCDM, WfGD and the ISP or (CMS), the applicable Receiving Water Quality Objectives (RWQO) and Reserve Determinations**, as well as the timeframes for short, medium and long terms measures in relation to the activity.

Step 2
This step is followed by written confirmation of the risk categorisation and classification of the activity or proposed water use. The risk categorisation for non-consumptive water uses, such as **section 21 (c) and 21 (i)** water uses, should be done in case there are other water uses associated with the activity.

Step 3
The next step in the IWWMP process entails information gathering. Information is obtained from other environmental authorisation processes conducted, such as the **EIA and EMPR** processes. The supporting specialist investigations together with the public participation process should provide the required input into the IWWMP. Should any information gaps exist in the available information, further specialist investigations should be conducted to supply the required information. The available information is utilised to document a broad project description and the baseline environmental situation. The relevant water use and waste management related legislative framework is summarised in the document as part of the legal assessment.

Step 4
This is then followed by a site characterisation or analyses phase. Large sites are delineated into smaller facilities to facilitate proper water use and waste related assessment. During this phase a detailed analyses is conducted of the water use and waste management activities on site, the operational management and the monitoring and controls implemented. It also includes an assessment of the implementation of best practices on site. The performance of a risk assessment is a critical component of the site characterisation. The risk assessment should address the aspects, impacts, and the severity and probability of the risks related to the activity. The identification of the high risks associated with the activity and the site characterisation process leads to the identification of the matters which require attention in an activity (problem statement) and also a statement relating to the adequacy of the available information.

The most important component of the IWWMP development process is the formulation of various strategies, goals and objectives for the water use or waste management of an activity, in accordance with the set philosophies and policies. The policies must address the four key areas related to IWWMP development, namely process water, storm water, ground water and waste. A range of management measures are then identified to reach the set goals and objectives. These management measures are then documented in an IWWMP action plan and this forms the heart of the IWWMP.

The IWWMP document is concluded with a statement on the legal status of the activity's water use and waste management and whether authorization is required, a motivation in terms of section 27(1) of the South African National Water Act (Act 36 of 1998) supported by key commitments of the water user to fulfil the aspects of section 27(1) of the SA NWA. The completed IWWMP is submitted to the relevant DWS Regional Office together with the completed and signed water use licence application forms, Title Deeds, the licence application fee, a copy of the Reserve Determination (if available), correspondence and the minutes of the IAP meeting(s).

The implementation of the IWWMP is an interactive process whereas its performance is monitored on an annual basis. The assessment of the IWWMP document itself, as well as the submission of information relating to monitoring and auditing conducted in terms of it could lead to the identification of its shortcomings, which must be addressed in the annual update of the action plan of the IWWMP. This will ensure that the concept of continual improvement is applied throughout the life cycle of the activity. As part of the IWWMP process the various *Roles and Responsibilities* of the different role-players need to be understood and respected. In all instances the point of entry for any departmental discussion is the relevant Regional Office. The National Office can be consulted to provide support and advice to the Regional Office on water use activities, although they should not be approached directly. They can be contacted through the relevant DWA Regional Office. It should be noted that the role of DWS is not to identify and select the water and waste management measures for implementation by a water user, as it is the responsibility of the water user to demonstrate to the Department that the selected management measures in the IWWMP action plan adhere to the "SMART"- concept i.e.:

S = sustainable;
M = measurable;
A = achievable;
R = resources allocated and
T = timeframe specific.

2.3.3.2 Contents of the Integrated Wastewater Management Programmes (IWWMP)

Additionally the manner in which the IWWMP is laid out could be adjusted to incorporate the aquatic toxicity tests to ensure that there is a standardised approach which would see the requirements included in the relevant sections as **highlighted** below:

I EXECUTIVE SUMMARY

II MAIN DOCUMENT
1 Introduction
1.1 Activity Background
1.2 Contact Detail
1.3 Regional setting and location of activity
1.4 Property description
1.5 Purpose of IWWMP

2 Conceptualisation of activity
2.1 Description of activity
2.2 Extent of activity
2.3 Key activity related processes and products
2.4 Activity life description
2.5 Activity infrastructure description
2.6 Key water uses and waste streams

5.4.4 Bio monitoring
5.4.5 Waste monitoring
5.5 Risk assessment / Best Practice Assessment
5.6 Issues and responses from public consultation process
5.7 Matters requiring attention / problem statement
5.8 Assessment of level and confidence of information

6 Water and waste management
6.1 Water and waste management philosophy (process water, storm water, groundwater, waste)
6.2 Strategies (process water, storm water, groundwater and waste)
6.3 Performance objectives / goals
6.4 Measures to achieve and sustain performance objectives
6.5 Option analyses and motivation for implementation of preferred options (Optional)
6.6 IWWMP action plan
6.7 Control and monitoring
6.7.1 Monitoring of change in baseline (environment) information (surface water, groundwater **Ecological Bioassays** and bio-monitoring)
6.7.2 Audit and report on performance measures
6.7.3 Audit and report on relevance of IWWMP action plan

7 Conclusion
7.1 Regulatory status of activity
7.2 Statement on water uses requiring authorization, dispensing with licencing requirement and possible exemption from regulations
7.3 Section 27 motivation
7.4 Proposed licence conditions

8 References

9 Appendixes: Specialist studies

2.4 FRAMEWORK FOR MONITORING AND ASSESSMENT PROGRAMME

Although water quality monitoring with physical and chemical indicators differs in philosophy and techniques from monitoring with biological indicators, the approaches both rely on sound practice in environmental science, including the following (Australian and New Zealand Guidelines for Fresh and Marine Water Quality, 2000):

- explicit written definition of the sampling site, project objectives, a hypothesis and the sampling protocol that will support the work;
- the definition of sampling sites, sampling frequency, and spatial and temporal variability that will permit appropriate statistical methods to be used;
- rigorous attention to field and laboratory quality control and assurance;
- incorporation of a pilot study to test the sampling protocol and determine spatial and temporal variability.

Figure 2-4 outlines the basic steps involved in developing a program for monitoring and assessing both biological and physico-chemical aspects of water quality. The first step of the framework is determining the primary management aims. Determining these aims will enable stakeholders to develop an appropriate conceptual model of key ecosystem processes and interactions. By doing this they can identify assumptions

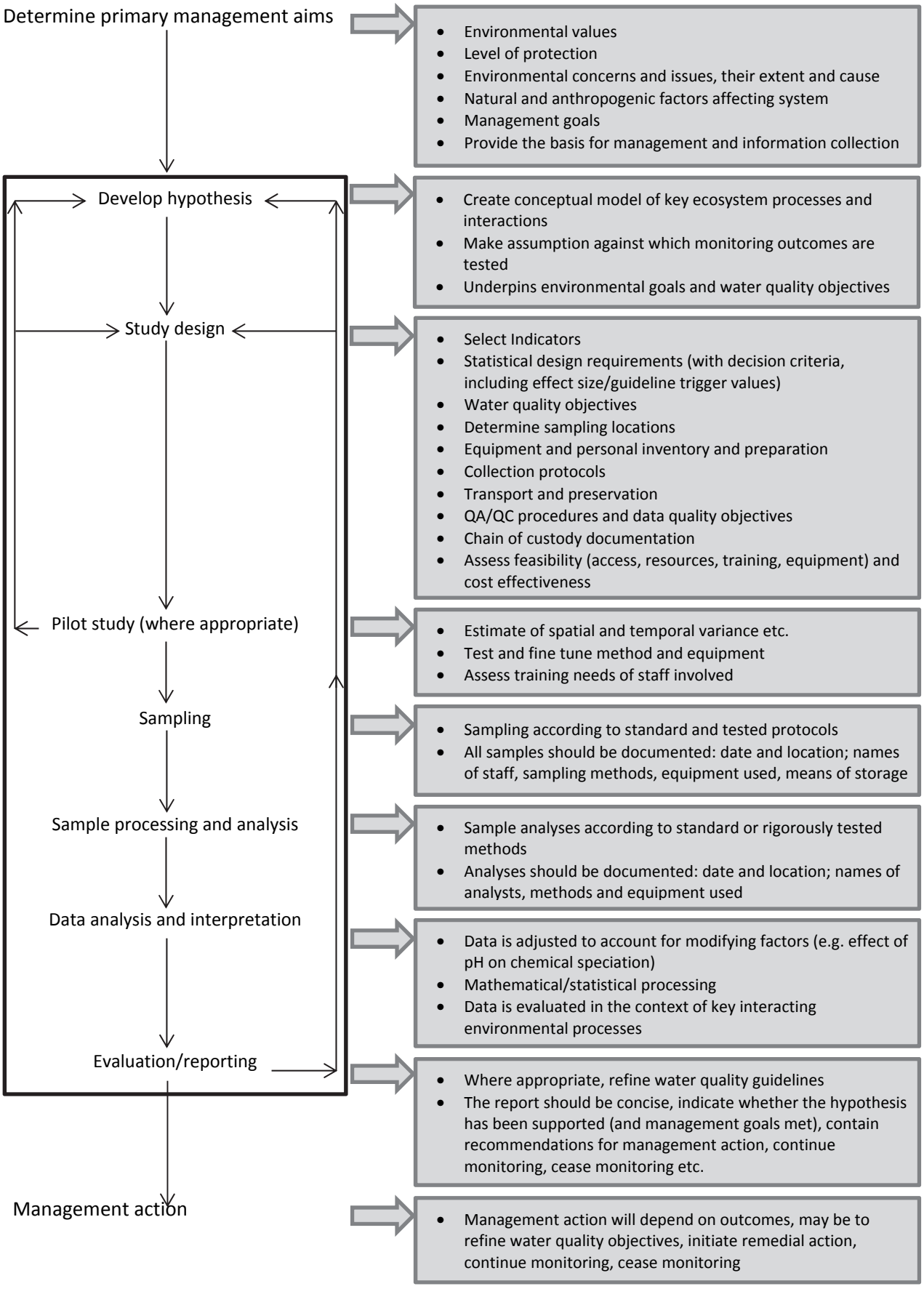

Figure 2-4: Procedural framework for the monitoring and assessment of water quality (Adapted from the Monitoring & Reporting Guidelines and the framework for designing a wetland monitoring program adopted by the Ramsar Wetland Convention in 1996).

against which monitoring outcomes can be tested, and develop appropriate working hypotheses whose predictions can be tested using the data that the program collects. Step two is developing a hypothesis.

This next step of the monitoring framework would include the selection of indicators and requirements for experimental design, including the determination of guideline values.

The monitoring programs identified should be maintained during and after implementation of the agreed management response(s), to evaluate their performance in achieving the water quality objectives and hence the management goals. This process should be iterative and on-going to ensure the environmental values continue to be reviewed and sustained.

CHAPTER 3: GUIDELINE DOCUMENT

3.1 INTRODUCTION

The guideline document and the associated Integrated Water Use Authorisation Bioassay Toolkit have been developed to assist license applicants and compliance monitors to correctly select industry specific water use license criteria. Workshops were held with government, industrial and consultant representatives in order to build an understanding of requirements as well as frustrations currently experienced with regards to the bioassay section of the water use authorisation process.

Currently this document and toolkit only address the approaches to be applied in the freshwater environment as well as the four major industries (Mining, Industrial, Agricultural and Municipal) which utilise these freshwater resources. As this is an initial approach to ensuring that applicable tests are used to assess relevant environmental issues, further studies will continue to refine the freshwater criteria as well as select methods for both estuary and marine environments. Furthermore, tests applicable for the smaller industries (e.g.: pharmaceutical industry, hospitals and personal hygiene) will be evaluated**.**

In recent years it has been recognised that pollution-related issues should be addressed by approaching the conservation, management and use of water resources in a holistic manner, according to the principles of integrated catchment management. Key strategies for achieving ecologically sustainable development include the involvement of stakeholders in decision-making processes and the development and adoption by industry of best management practice guidelines.

Both the quality and the quantity of water resources are critical issues for industry, mining, municipal, agriculture and aquaculture in South Africa. Water quality is also of major importance for the protection of human consumers of food products. Growth of these major primary industries, together with expanding urbanisation and other industrial development, has increased the demand for good quality water but at the same time exerted escalating pressures on the quality of the water resources that are available. Therefore, to assess water quality for primary industries, not only must productivity issues be considered but also the possible adverse effects of these enterprises on downstream water quality and activities. These contributing factors were therefore integrated into a framework (Figure 3-1) to be used to generate industry and site specific license conditions.

The South African NWA was carefully examined to identify contexts that could potentially benefit from information from toxicity tests. Considerable insight was obtained during discussions on how DWS approaches the implementation of the South African NWA during the Slabbert, Murray WRC project (WRC, 2011). For example, much emphasis is placed on the nature of the water use, e.g. as categorised in the South African Water Quality Guidelines. Water uses typically define target systems (*i.e.* those affected by toxicity like humans, animals, etc.), which provide a useful link with effect-based thinking. The Water Quality Guidelines were thus also examined for inputs into the information requirements. While perusing the South African NWA and Water Quality Guidelines various generic water sources were identified that could serve as origins of samples for toxicity tests. A series of management contexts were identified that would require toxicity test information, involving both the identified NWA contexts and the generic water sources. In addition, a series of management criteria were identified and defined. These included generic management criteria and other criteria.

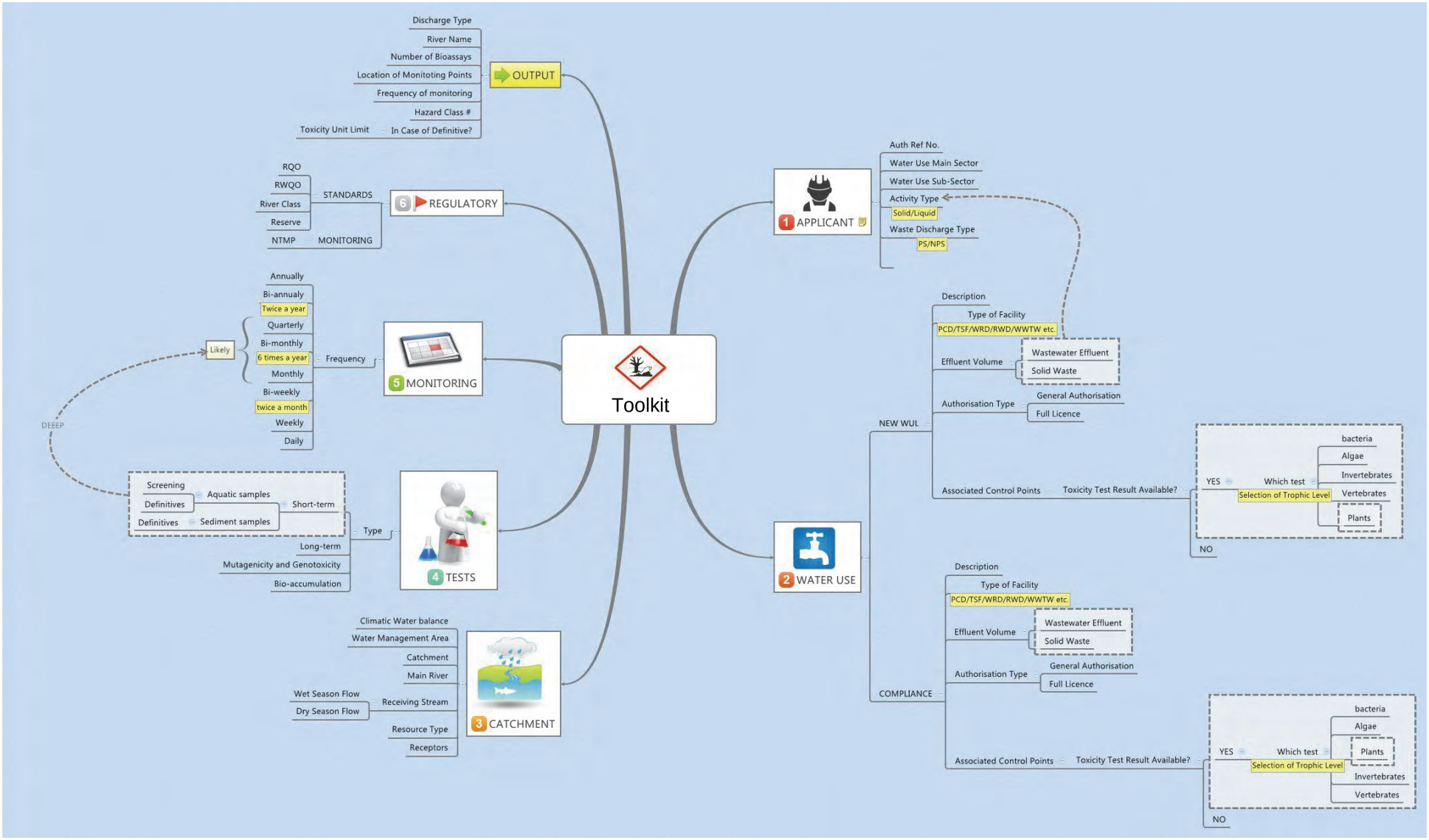

Figure 3-1: Water Use Authorisation Bioassay Toolkit layout

Classification options associated with each generic management criterion were also identified. Finally, each management context was allocated an appropriate classification for each generic management criterion (WRC 2010).

Therefore, to assess water quality for primary industries, not only must productivity issues be considered but also the possible adverse effects of these enterprises on downstream water quality and activities. The quality of the water can affect water uses or the health of aquatic ecosystems in different ways. The effect of effluents therefore needs to consider the impact on the health of individuals drinking the water or swimming in it as well as productivity or yield of a crop being irrigated. The quality of water can additionally affect the cost of treating water or the sophisticated technology needed to treat the water to an adequate quality even before it is used in an industrial process. Whenever water quality degradation takes place as a result of poor quality effluent being released into water resources it impacts the biodiversity of an aquatic ecosystem. It is therefore important to use the different criteria such as health effects, crop quality, cost of treatment; type and level of treatment technology; and the effects on biodiversity as determinants for making decisions about the fitness for use of water (DWAF, 1996).

3.2 INTEGRATED WATER USE AUTHORISATION BIOASSAY TOOLKIT

Although bioassays have been included in Water licenses to assess and monitor an environmental effect, the wording, requirements and clarity have resulted in confusion and inconsistent license requirements. The Integrated Water Use Authorisation Bioassay Toolkit (Figure 3-2) is intended to provide guidance on site specific wording for the inclusion of bioassays into Water Use Authorisations.

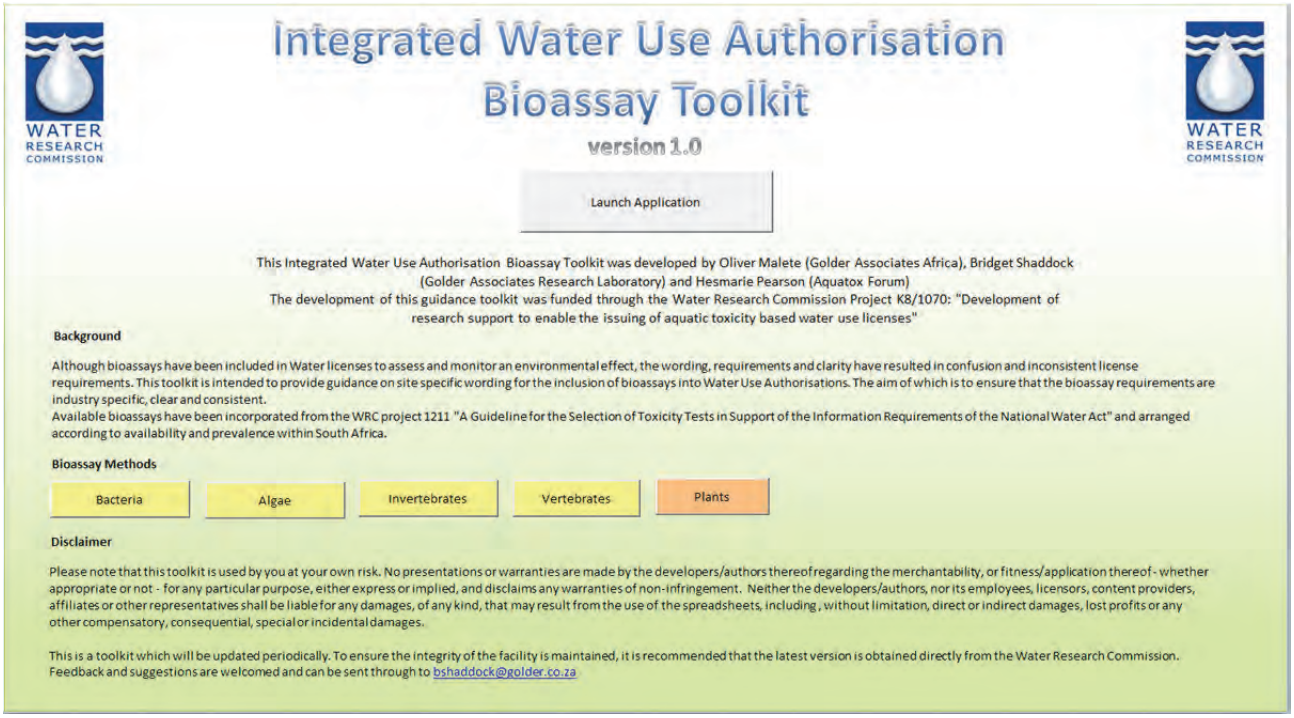

Figure 3-2: Integrated Water Use Authorisation Bioassay Toolkit launch page

The aim of the toolkit is to ensure that the bioassay requirements for the license conditions are industry specific, clear and consistent. This toolkit integrates the standard Water Use Authorisation information to populate and generate the conditions. The output of the toolkit will include license conditions as well as an output which can be stored. With additional refinement of this toolkit, the requirements as well as river conditions will be uploaded onto a central database, contributing to management strategies and resource quality objectives.

Once launched, the toolkit takes the user through successive screens to gather the necessary information and generate the license conditions.

3.2.1 SECTION A: APPLICANT INFORMATION

This section of the toolkit captures the relevant information of the water use applicant in order to customise the bioassay wording. The main sector and subsector of the applicant as well as the water use type provides information on the potential contributing effect of the water use applicant.

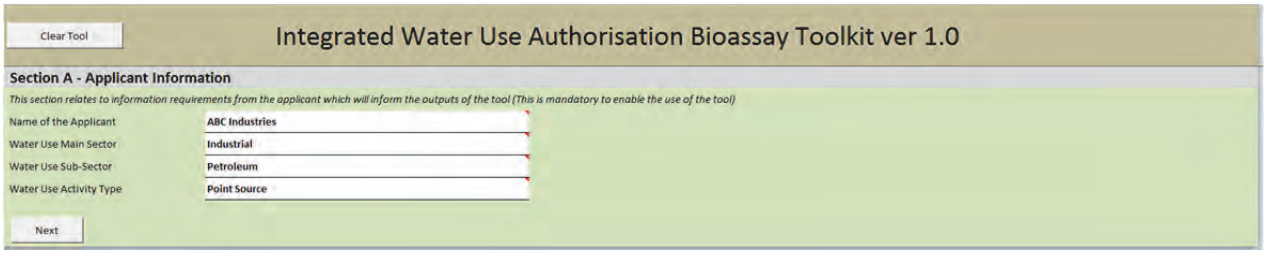

Figure 3-3: Section A – Applicant information example

3.2.2 SECTION B: DESCRIPTION OF WATER USE

The description of the water use relates to whether or not the toolkit is being used to generate wording for a new water use authorisation or an existing water use authorisation where ongoing compliance needs to be updated. The toolkit users which have previous licenses will be able to capture the license information which should be associated with the renewal process.

- Section B1: New Water use Authorisations
- Section B2: Ongoing compliance

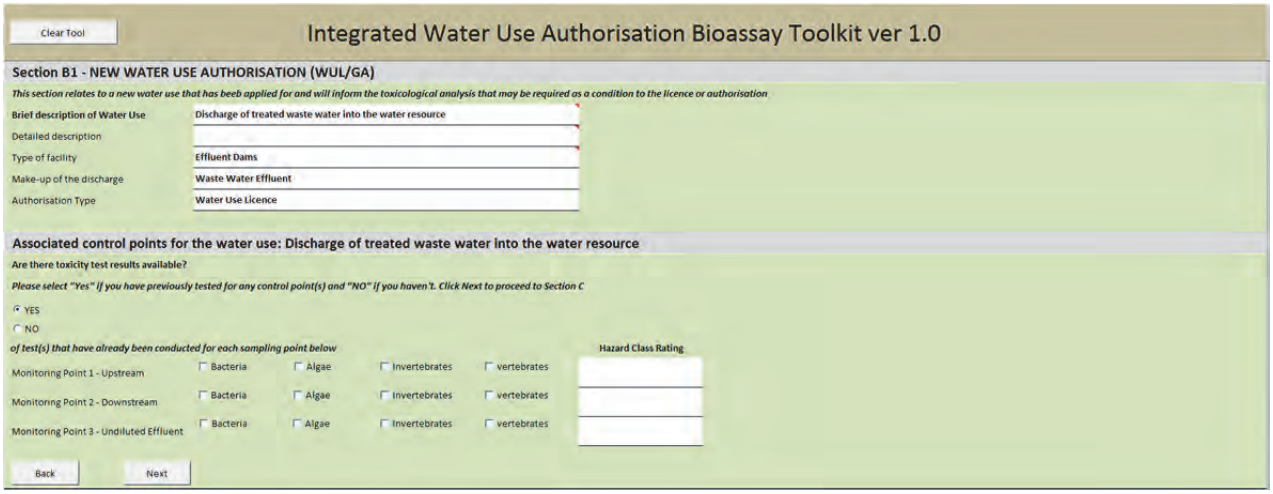

Figure 3-4: Section B1 – Water Use Authorisation example

3.2.3 SECTION C: CATCHMENT AND RIVER INFORMATION

The catchment and river information is used to generate resource specific conditions as well as capture known river conditions. This information captured on the output page can be kept for historical purposes as well as traceability for the license application. Any alterations in the river health conditions over time will therefore be captured and translated into the resource requirements for the license conditions.

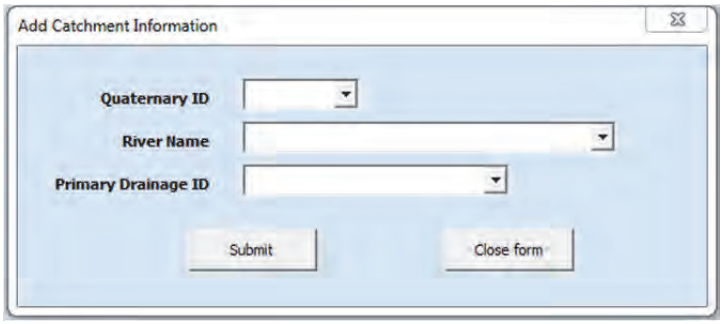

Figure 3-5: Catchment information input screen

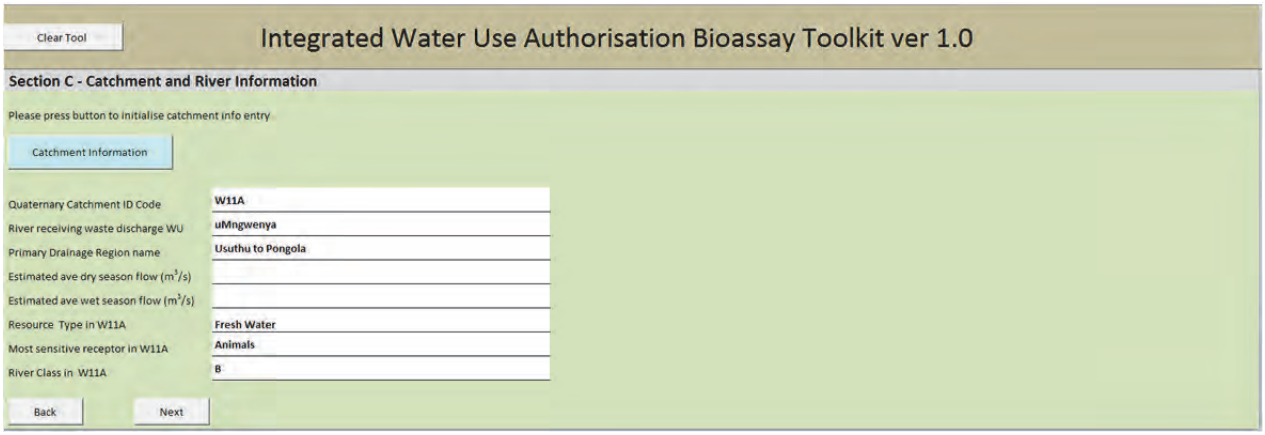

Figure 3-6: Section C – Water Use Authorisation example

3.2.4 SECTION D: LABORATORY TESTS

One of the main purposes of the toolkit is to generate the required wording as well as guide consultants and industry professionals in the selection of environmental bioassays. The selection of environmental bioassays seems daunting; therefore this section gathers information on previous environmental bioassay results as well as known interferences in samples which could prevent a water use applicant from complying with the licence conditions. Rather than placing unrealistic requirements in place should interferences exist, the toolkit will generate advice on how to address these challenges.

Bioassay databases have been incorporated from the WRC project 1211 "A Guideline for the Selection of Toxicity Tests in Support of the Information Requirements of the National Water Act" and arranged according to availability and prevalence within South Africa. The list of these bioassays will be accessible from both the output screen as well as the launch page.

Bioassays are living organisms which have been shown to give representative results on possible environmental effects. The various trophic levels (bacteria, algal, invertebrates, vertebrates, plants) which have been incorporated into standard DEEEP assessments represent the levels of the food web where changes could occur due to alterations in the health of the water resource.

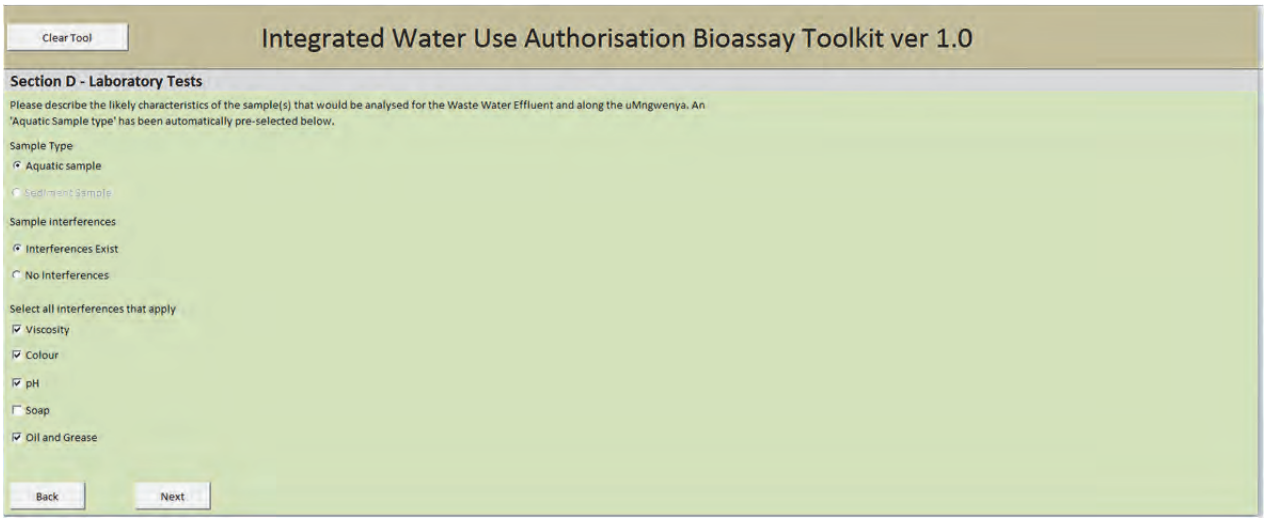

Figure 3-7: Section D – Laboratory test characteristics example

3.2.5 SECTION E: TOOLKIT OUTPUT

The generated output (Figure 3-8) from the Toolkit outlines the applicant and related discharge type, required bioassays, minimum sampling frequency as well as hazard class requirements. If the samples were identified with potential interferences, the additional advice on how to address these challenges is included below the bioassay conditions for the water use application.

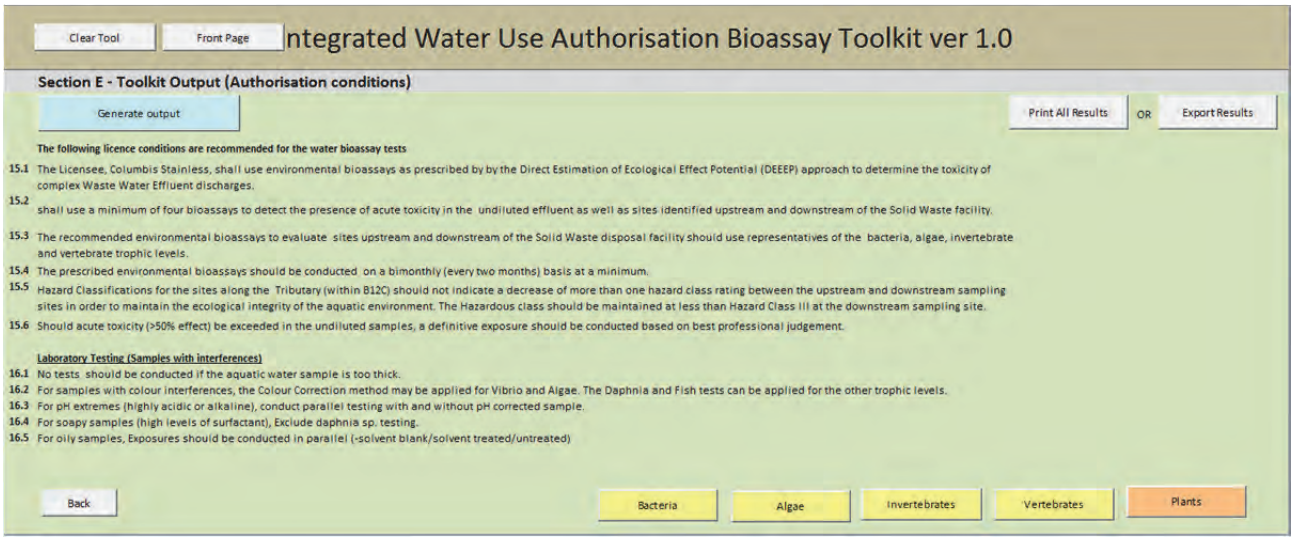

Figure 3-8: Section E – Toolkit Output example

Information on the recommended bioassay tests can be accessed from the output screen as well as the toolkit launch page. Once the Authorisation conditions have been generated, the inputs and output can be printed and saved for future use. With further development, the aim would be to upload the output information into a database which would be incorporated into the toolkit conditions.

3.3 CONCEPTUAL INFORMATION

An important first step in using this guideline document is to consider the management framework for their application. This includes defining the primary management aims, determining appropriate trigger values, defining water quality objectives, and establishing a monitoring and assessment program to address these

objectives. The type of monitoring and assessment program required will be specific to each situation, but there are several broad principles or procedures that are common to all programs. For details refer back to Figure 2.4 which gives a generic flow chart of the procedural framework for monitoring and assessment.

The Water Use Authorisation Bioassay Toolkit can be used for generating license conditions for the following applications:

- Wastewater/effluents/final discharges
- Process water
- Storm water run-off
- Surface water/receiving water/river water
- Groundwater
- Sediments
- Sediment pore water
- Waste dump leachates
- Soil leachates
- Extracts of solid wastes
- Chemicals and products
- Drinking water

However, it is important to note that the sampling procedure and sample preparation may be different for each of these groups.

3.3.1 INDUSTRIAL WASTEWATER

Water used for industrial purposes is subdivided into a number of subcategories such as water used for steam generation, cooling, lubrication, humidification, etc. Each of these subcategories of water use can each have different water quality requirements. Therefore guidelines for this type of water and effluent is characterised according to subcategories or components which specify water quality requirements at a sub-sector level (Table 3-1) (DWAF, 1996).

Table 3-1: Industrial Sector Information

Main Sector	*Sub-sector*
Industrial	Petroleum
	Paper and Pulp
	Power
	Textile

3.3.2 MINING EFFLUENT

During mining operations minerals are extracted from underground mines, surface mines and quarries using machinery and explosives. During these extraction processes large quantities of water are used. Often water is additionally used after extraction when the raw minerals are subject to crushing, milling, washing and chemical treatments. Mining effluent is therefore generated during mineral processing (e.g., stone cutting, wash water, scrubber water), from equipment cooling, from mine dewatering, and from storm water runoff both at the mines as well as at the processing plants. The various mining sectors (Table 3-2) within South Africa result in varying degrees and exposure routes for contaminants to enter the aquatic ecosystems.

Table 3-2: Mining Sector Information

Main Sector	*Sub-sector*
Mining	Gold Mines
	Platinum Mines
	Coal Mines (Open cast)
	Copper Mines

3.3.3 MUNICIPAL WASTEWATER

The sewer system collects wastewater from homes, businesses and many industries; this sewer waste is then delivered to a wastewater treatment works for treatment. These wastewater treatment works have been built with the primary aim of cleaning the wastewater for discharge into streams or other receiving waters, or for reuse. Continuing industrial and commercial growth in many countries around the world in the past decades have been accompanied by rapid increases in Municipal Solid Waste (MSW) and Industrial Solid Waste (ISW) generation. South Africa has a vast system of collection sewers, pumping stations, and treatment works. At present, landfilling is the most popular way of solid waste disposal and landfilling will continue to be the primary means of MSW and ISW disposal in future. Besides scarcity of available landfill sites in certain regions, a large amount of leachate (originating from water which has percolated through emplaced refuse) generated from a landfill site poses a major problem of landfill disposal of MSW and ISW. For the municipal sector (Table 3-3) the proper treatment of wastewater treatment works effluent as well as leachate has therefore become a challenging problem for local authorities (Schoeman et al., 2003).

Table 3-3: Municipal Sector Information

Main Sector	*Sub-sector*
Municipal	Wastewater Treatment Works
	Waste Dumps
	Storm Water

3.3.4 AGRICULTURAL EFFLUENTS

Irrigation and livestock watering are the major agricultural uses of water. Minor amounts are used for other production purposes, such as the mixing of pesticide, fertiliser and veterinary formulations, and livestock dietary supplements. Both the irrigation and livestock industries rely heavily on the use of groundwater, as well as surface water resources. Thus the guidelines provided for these industries (Table 3-4) are applicable (where appropriate) to both surface and groundwater quality.

Table 3-4: Agricultural Sector Information

Main Sector	*Sub-sector*
Agricultural	Live Stock
	Crops
	Aquaculture
	Processing Plants

3.3.5 HEALTH EFFLUENTS

Although the current toolkit does not provide in-depth approaches for the health industry (Table 3-5), preliminary conditions have been included which will be updated in further revisions.

Table 3-5: Health Sector Information

Main Sector	*Sub-sector*
Health	Hospitals
	Medicine

3.3.6 SAMPLE POINTS

Water use licenses are issued in order to allow industries to utilise water or release waste into back into a water resource. For these reasons sampling points need to be selected in order to evaluate impacts. First and foremost the effluent/outflow or leachate should be assessed undiluted so as to monitor the effect of the "concentrated" contaminant source (CS). In order to assess the effect of a water user on the receiving environment samples should be collected from a site upstream (within 5 km radius) of an identified point of potential impact (point source or no-point). This sampling point is used to assess the resource before the contaminant enters and is therefore "unimpacted", therefore there should not be any other potential sources of impact between this sampling site and the potential impact being monitored. Additionally a sample should be collected from a site downstream (within 5 km radius of discharge) of the identified point of potential impact in order to assess the influence of the industry on the receiving environment in comparison to the bioassay results obtained from the unimpacted site upstream. This sampling point approach allows for problematic outflows to be identified and remediation measures applied.

If more than one potential source of contamination is identified then each source should be assessed in this manner, however it should be noted that if potentially contaminated sources are all entering the same aquatic resource, then the downstream site of a previous contaminant source being evaluated can often represent the upstream site of the successive impact point (Figure 3-9). In this way sampling and testing costs can be minimised and all potential impacts can be assessed.

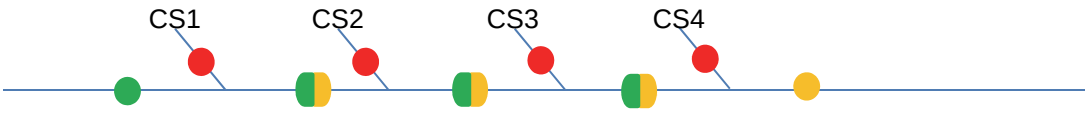

Figure 3-9: Sampling point locations with outflow sites indicated in red, upstream sites indicated in green and downstream sites indicated in orange

3.3.7 SAMPLING FREQUENCY

It is recommended that sampling should be done under conditions of low flow in the receiving water so that worst case toxicity conditions become apparent. It is very important to closely replicate the worst case receiving water conditions in toxicity tests, because of the influence of environmental conditions. Seasonal alterations in the quality of the effluent as well as the aquatic resource should be addressed. Therefore for the first two years sites should be monitored a minimum of four times a year (quarterly). After this initial monitoring period, only if no toxicity has been observed, can the monitoring schedule be altered to twice a year (biannual). In cases where seasonal variation in toxicity is been observed, the reason for the toxicity must be addressed.

In industries where the quality of effluent should be controlled or is associated with seasonal maintenance, the frequency of sampling may be more frequent (e.g. monthly, before or after seasonal maintenance, etc.).

3.3.8 TEST BATTERY

Due to the large number and variety of contaminants present in the aquatic environment, the analysis required to identify the potential for impact can be both expensive and time consuming. Therefore in order to achieve a realistic estimation of the hazard of these contaminants it is necessary to know their toxic effects. In order to compare the standard chemical analyses with bioassay results, it is necessary for environmental samples to be assessed using a battery of tests as opposed to a single specie test. Such batteries allow for information about the whole ecosystem to be quantified, which makes it easier to assess the real hazard in the environment.

A standard battery of tests normally consists of representative species from various trophic levels:

- Fish (e.g. guppy (*Poecilia reticulata*) or Zebra fish (*Danio rerio*))
- Invertebrates (e.g. Crustacea (*Daphnia pulex* or *D. magna*))
- Algae (e.g. Green alga (*Selenastrum capricornutum*))
- Bacteria (e.g. *Vibrio fischeri*)

3.3.9 HAZARD CLASSIFICATION

Although the growing awareness for the impact of hazardous discharges/effluents and wastes in aquatic and terrestrial environments has triggered preventive as well as remedial action, the degree of environmental pollution has to date not been mapped on a site by site basis in detail in many countries (Persoone et al, 2003).

The approach that is used in the first instance to determine the level of contamination of surface waters, sediments or soils, is the quantitative analysis of the chemical compounds presumed to be present. Although chemical analyses give a preliminary insight into the importance of the hazard to the receiving biological systems, this approach, as emphasised in literature, suffers from two major limitations: a) the restriction of the analyses to the chemical compounds (or groups of compounds) prescribed by environmental legislations and b) the interactions and/or the bioavailability of the contaminants. The former drawback automatically eliminates from the hazard evaluation every compound that is “not looked for”, whereas the latter often leads to either an over- or an under-estimation of the real danger (Persoone et al, 2003).

To date attention is therefore also paid to the “biological” approach to find out the hazard resulting from the combined impact of “all” the pollutants discharged or found present. Biological indexes have been worked out and biological monitoring of surface waters are performed “in space and in time” in various countries to qualitatively and quantitatively compare the fauna and flora found with those that are normally expected to colonize the water bodies. The problem with monitoring with the aid of biological indexes is that the outcome reflects the impact that pollutants have exerted on the indigenous biological communities “during a period of unknown duration” (Persoone et al, 2003).

In order to find out the impact caused by accidental or voluntary releases of toxicants, or the level of biological hazard of contaminated sites “at a particular moment in time”, a second and as important aspect of the biological approach, namely toxicity testing or environmental bioassays, is therefore needed. These bioassays with selected species representative of the trophic chain of production – consumption – degradation signal the effects of all the pollutants present “at the very moment of sampling” (Persoone et al, 2003).

Ecotoxicological testing is presently advocated in the environmental legislation in many countries to quantify the degree of toxicity of industrial effluents, wastewaters and/or solid wastes and to set limits for their discharges, as well as to determine whether and to what extent aquatic or terrestrial sites are toxic to biota (Persoone et al, 2003).

In order to provide an ecological hazard assessment there is a need for assessment criteria. These criteria would generally be numerical values with which the test results can be compared to pronounce on the expected impact implied by the test result.

For the purpose of this toolkit the Acute Hazard Classification System of Persoone et al. (2003) has been included information gathering purposes. Additional Hazard Classification background information is available in Appendix E.

After determination of the percentage effect (hereafter referred to as “PE”) obtained with each of the bioassays, the water is ranked into one of the following 5 classes on the basis of the highest toxic response shown by at least one of the tests applied:

	Class	***Hazard***	***Percentage Effect***
	I	No acute hazard	None of the tests show a toxic effect (i.e. an effect value that is significantly higher than that in the controls).
	II	Slight acute hazard.	A statistically significant PE is reached in at least one test, but the effect level is below 50%.
	III	Acute hazard.	The 50% Percentage Effect (PE50) is reached or exceeded in at least one test, but the effect level is below 100%.
	IV	High acute hazard tolerant taxa present.	The PE100 is exceeded in at least one test.
	V	Very high acute hazard.	The PE100 is exceeded in all tests.

CHAPTER 4: REFERENCES

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APPENDIX A: QUALITY ASSURANCE

A.1. Quality

In order to ensure that the recommendations put forward for inclusion into license conditions provide the level of information needed to protect the aquatic resources, it is necessary to provide guidance on the recommended quality control which should be applied. How the samples are collected and analysed will ensure comparable and reliable results. The information provided below is aimed at consultants and technicians implementing the recommended tests as well as the compliance officers monitoring the requirements of the license.

A.2. Quality Assurance Programs

Laboratory accreditation provides a means for third-party certification of the competence of laboratories to perform specified types of testing and calibration. These capabilities must be periodically evaluated (measured) according to the requirements contained in ISO/IEC 17025:2005. This serves to maintain confidence in the laboratory's ability to perform accurate and valid measurements and tests.

A.2.1 South African National Accreditation System (SANAS)

SANAS is an independent body capable of assessing organisations and laboratories for compliance to the relevant international or national standards and verifying their competence for tasks undertaken within the scope of their activities. Laboratories receiving SANAS accreditation benefits from the impartial assessment of their performance by experts. SANAS has its office on the Department of Trade and Industry (DTI) Campus, Sunnyside, Pretoria, South Africa and is directed and legally represented by a Board of Directors whose members are appointed by the Minister of Trade and Industry. SANAS operates in accordance with the requirements, criteria, rules and regulations laid down in the following documents:

The requirements of the international standard ISO/IEC 17011, the general requirements for bodies providing assessments and accreditation of conformity assessment bodies. The requirements as stipulated in the various Memorandums of Agreement with the international bodies and the national regulatory bodies.
The Accreditation for Conformity Assessment, Calibration and Good Laboratory Practice Act, 2006 (Act 19 of 2006). “To provide for an internationally recognised and effective accreditation and monitoring system for the Republic by establishing SANAS as a juristic person; to recognise SANAS as the only accreditation body in the Republic for the accreditation of conformity assessment and calibration and monitoring of good laboratory practice; and to provide for matters connected therewith.”

Note: SANAS documentation manuals, accreditation process, training courses and fees are available free of charge from the SANAS web site: www.sanas.co.za.

SANAS accreditation gives formal recognition that laboratories are competent to carry out specific tasks. Organisations accredited by SANAS become a stakeholder in SANAS and are entitled to use the appropriate SANAS logo on the certificates they issue, their letterheads and promotional material. Formal recognition of the competence of a laboratory by an accreditation body in accordance with international criteria has many advantages:

- Potential increase in business due to enhanced customer confidence and satisfaction in meeting their demands.
- Savings in terms of time and money due to reduction or elimination of the need for re-testing of products.
- Better control of laboratory operations and feedback to laboratories as to whether they have sound quality assurance systems and are technically competent.

- Increase of confidence in testing data and personnel performing tasks.
- Customers can search for and identify the laboratories accredited by SANAS for their specific requirements from the SANAS website.
- Users of accredited laboratories will enjoy greater access to their products in both domestic and international markets when tested by accredited laboratories.

Increasing emphasis is currently being placed on the responsibility of manufactures for the environmental impacts of their products from “Cradle to Grave”. Using an accredited laboratory with internationally accepted test method will be legally defensible, e.g. in cases where accredited test methods are used to monitor point source pollution of environmental waters and setting license conditions. South Africa’s aquatic toxicity test results will be on par with results in countries like the USA and Canada that pioneered the application of aquatic toxicity tests to prevent pollution and protect the environment.

In South Africa these capabilities must be periodically evaluated (measured) according to the requirements contained in ISO/IEC 17025:2005. Laboratories receiving SANAS accreditation benefits from the impartial assessment of their performance by experts. This serves to maintain confidence in the laboratory's ability to perform accurate and valid measurements and tests.

A2.2. Quality assurance and quality control (QA/QC)
Quality assurance and quality control (QA/QC) procedures should be part of any sampling protocol. Quality control (QC) and quality assurance (QA) are different but related concepts. In the context of these Guidelines, *quality control* means devising and implementing safeguards to minimise the corruption of data. These safeguards must be installed at every step of the process from project definition to the decision on whether measured concentrations compare acceptably with the guidelines. *Quality assurance* means testing the effectiveness of these safeguards.

In any QA/QC program, chain of custody documentation is essential to ensure that errors can be traced. Chapter 4 of the Monitoring Guidelines discusses QA/QC in some depth for key points for chemical, physical and toxicant indicators.

A specific formal statement of quality control for physical and chemical indicators is this:
The overall objective of quality control in the measurement of physical and chemical variables is the determination of the *exact* indicator concentration that existed at a specifically defined location at the time the sample was taken. In most cases this requirement extends to the chemical speciation of the indicator.

Neglect of QA/QC is probably the most important reason for the unreliability of most historical chemical data. Protocols for field and laboratory aspects of sampling must be followed carefully, as discussed in the Monitoring Guidelines. QA/QC begins with the choice and training of competent field and laboratory staff; it includes the choice and maintenance of field and laboratory equipment and vehicles. It extends to the checking of analytical methods and analytical performance, the tracking of each sample throughout sampling and analysis, and the accurate recording of data in the final database.

There is an increasing demand, driven either by legislation or regulatory requirements for QA and QC in biological tests (Hale, 1998). Toxicity tests used for regulatory compliance must provide the same results when applied for the same effluent/chemicals in different laboratories as well as for tests performed in the same laboratories at different times of the year.

QA practices within an aquatic toxicology laboratory must address all activities that affect the quality of the final effluent toxicity data, such as:

- effluent sampling and handling;

- the source and condition of the test organisms;
- condition and operation of equipment;
- test conditions;
- instrument calibration;
- replication;
- use of reference toxicants;
- record keeping; and
- data evaluation.

A.2.3. Method validation and precision

Like all measurements toxicity tests exhibit variability, Factors such as the test organism age, condition, sensitivity, temperature control, salinity, pH control, etc. can effect precision. Quality assurance practices should, therefore, be established. The use of a standard control water and the inclusion of a reference toxicant with test procedures is recommended. Minimum criteria of test acceptability specific for each endpoint that is measured in the controls should also be established (US EPA, 1991a).

It is a laboratory's responsibility to demonstrate its ability to obtain consistent, precise results with reference toxicants before it performs toxicity tests with effluents for compliance purposes. To meet this requirement, the intra-laboratory precision, expressed as percent coefficient of variation (CV%), of each type of test to be used in a laboratory should be determined by performing five or more tests with different batches of test organisms, using the same reference toxicant, at the same concentrations, with the same test conditions (i.e., the same test duration, type of dilution water, age of test organisms, feeding, etc.), and same data analysis methods. A reference toxicant concentration series (0.5 or higher) should be selected that will consistently provide partial mortalities at two or more concentrations.

<u>On-going laboratory performance</u>

According to US EPA (2002) satisfactory on-going laboratory performance is demonstrated by performing at least one acceptable test per month with a reference toxicant for each toxicity test method conducted in the laboratory during that month. For a given test method, successive tests must be performed with the same reference toxicant, at the same concentrations, in the same dilution water, using the same data analysis methods. Precision may vary with the test species, reference toxicant, and type of test. Each laboratory's reference toxicity data will reflect conditions unique to that facility, including dilution water, culturing, and other variables; however, each laboratory's reference toxicity results should reflect good repeatability.

Control charts should be prepared for each combination of reference toxicant, test species, test condition, and endpoint. Toxicity endpoints from five or six tests are adequate for establishing the control charts. In this technique, a running plot is maintained for the toxicity values (Xi) from successive tests with a given reference toxicant, and endpoints (EC/LC_{50}'s) are examined to determine if they are within prescribed limits. The outliers, which are values falling outside the upper and lower control limits, and trends of increasing or decreasing sensitivity, are readily identified. At the P0.05 probability level, one in 20 tests would be expected to fall outside of the control limits by chance alone. If more than one out of 20 reference toxicant tests fall outside the control limits, the laboratory should investigate sources of variability, take corrective actions to reduce identified sources of variability, and perform an additional reference toxicant test during the same month. In those instances when the laboratory can document the cause for the outlier (e.g., operator error, culture health or test system failure), the outlier should be excluded from the future calculations of the control limits. If two or more consecutive tests do not fall within the control limits, the results must be explained and the reference toxicant test must be immediately repeated. Actions taken to correct the problem must be reported.

If the toxicity value from a given test with the reference toxicant falls well outside the expected range for the test organisms when using the standard dilution water, the laboratory should investigate sources of

variability, take corrective actions to reduce identified sources of variability, and perform an additional reference toxicant test during the same month.

Proficiency Testing Scheme (PTS)

Interlaboratory precision is the ability to obtain consistent results repeatedly when doing a specific test with the same reference toxicant while intralaboratory precision (round robin tests) indicates how reproducible a method is when carried out by different laboratories using the same test and reference toxicant.

A PTS is a powerful quality assurance tool for analytical measurement laboratories.

Samples are distributed to laboratories for analysis, the results evaluated and a report generated.
Laboratories should use their routine methods of analysis.
Proficiency should be used as an educational tool to identify and address measurement problems.

Benefits of proficiency testing include:

- Provides a regular independent check on the quality of analytical measurements.
- Enables participants to compare their performance with peer laboratories.
- Offers constructive feedback from the Technical Committee (TC).
- Facilitates the demonstration of competence to accreditation bodies, regulators and customers.
- Enables monitoring of trends, over time, in the quality of measurements.
- Assists in the evaluation of methods and instrumentation.
- Helps educate laboratory staff and their customers.
- Promote improvements in the method of analysis.
- Provide laboratories with a means of objectively assessing and demonstrating the reliability of their analysis.
- Provide information on the field performance of the method.

Many international and national standards emphasise the importance of maintaining environmental quality. To ensure a safe environment, analytical results are frequently used as a measuring tool and have to be accurate and reliable. Participation in a proficiency testing scheme (PTS) provides the opportunity to measure the quality of analysis. It also helps laboratories to meet the requirements of accreditation organisations and assists laboratories to optimise their quality control and analytical performance for the benefit of their customers. It provides independent evidence of laboratory performance for both laboratory management and customers.

Although using an accredited laboratory is preferred, it is a minimal recommendation that the laboratory used should participate in a recognised PTS, such as the Acute Toxicity PTS run by the National Laboratory Association (NLA). The scheme will be based on the requirements of ISO/IEC 17043:2010, Edition 1; Conformity assessment – General requirements for Proficiency testing, and will include the following toxicity tests:

• 30 minute *Vibrio fischeri* bioluminescent bacteria toxicity test.
• 72 hour *Selenastrum capricornutum* growth inhibition test.
• 48 hour *Daphnia pulex* and/or magna acute toxicity test.
• 96 hour *Poecilia reticulata* and/or *Danio rerio* acute toxicity test.

The significance of these aquatic toxicity tests is to help in the assessment of possible risk to similar species in the natural environment and as an aid in determination of possible water quality criteria for regulatory purposes for use in correlation with acute testing of other species for comparative purposes (US EPA, 2002).

Aquatic toxicity tests, such as mentioned above, are applied to assess water pollution and are primarily used to screen for toxic substances in the aquatic environment and to some extend to predict the toxic effect of environmental impacts on invertebrates. A further use of these tests is to comply with the “The Management of Complex Industrial Wastewater Discharges: Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach as introduced by DWAF (2003).

Laboratories wishing to join the scheme should complete the PT Scheme Participation Application Form (NLA-PT-F-04). Note that the individual indicated as the contact person, will receive all communications, including those concerning sample delivery, and the reports.

Record keeping

Proper record keeping is important. A complete file should be maintained for each individual toxicity test or group of tests on closely related samples. This file should contain a record of the sample chain-of-custody; a copy of the sample log sheet; the original bench sheets for the test organism responses during the toxicity test(s); chemical analysis data on the sample(s); detailed records of the test organisms used in the test(s), such as species, source, age, date of receipt, and other pertinent information relating to their history and health; information on the calibration of equipment and instruments; test conditions employed; and results of reference toxicant tests.

Laboratory data should be recorded on a real-time basis to prevent the loss of information or inadvertent introduction of errors into the record. Original data sheets should be signed and dated by the laboratory personnel performing the tests.

A.3. SAMPLES AND SAMPLING

A.3.1. Sampling points

A.3.1.1. Effluent

An effluent or in-stream sampling programme is important to obtain a sample from which a representative measure of the parameter of interest can be made. Effluent variability is an important factor to consider when selecting the method of sampling and the frequency.

Sampling must be tailored to measure the type of toxicity of concern for a particular discharge, for example long-term effects which are more constant or acute effects which are more variable and subject to peaks of intensity.

The effluent sampling point is ordinarily the same as that specified in the Water Use Licence (WUL. Conditions, there can however be exceptions if it is not possible to sample directly from this point. Alternative sampling points would be selected if:

- better access to a sampling point exists between the final treatment and the discharge outfall;
- the effluent is chlorinated prior to discharge to the receiving waters, it may also be desirable to take samples prior to contact with the chlorine to determine toxicity of the un-chlorinated effluent; or
- there is a desire to evaluate the toxicity of the influent to publicly owned treatment works or separate process waters in industrial facilities prior to their being combined with other process waters or non-contact cooling water, additional sampling points may be chosen.

A.3.1.2. Receiving water

The sampling point is determined by the objectives of the test. In rivers, grab samples should be collected at mid-stream and mid-depth, if accessible. At estuarine and marine sites, samples should be collected at mid-depth.

To determine the extent of the zone of toxicity in the receiving water downstream from the outfall, receiving water samples are collected at several distances downstream from the discharge, depending on budget

constraints. The time required for the effluent receiving-water mixture to travel to sampling points downstream from the outfall, and the rate and degree of mixing, may be difficult to ascertain. Therefore, it may not be possible to correlate downstream toxicity with effluent toxicity at the discharge point unless a dye study is performed. The toxicity of receiving water samples from five stations downstream from the discharge point can be evaluated using the same number of test vessels and test organisms as used in one effluent toxicity test with five effluent dilutions.

Logistical problems and difficulty in securing sampling equipment generally preclude the collection of composite receiving water samples for toxicity tests. Therefore, it is common practice to collect a single grab sample and use it throughout the test.

A.3.1.3. Mixing zone

A conservative approach to protect the aquatic environment against toxic effects is to require that an effluent has no observable toxicity prior to entering a receiving water. In practice it is often necessary to allow the receiving water to dilute a toxic effluent so that non-toxic levels occur in most of the receiving water. This means that the area of immediate discharge (mixing zone) will experience an effluent concentration which is toxic, and organisms in this area will be more or less severely affected.

One reason for designating a mixing zone would be to reduce treatment costs of a discharger in situations where rapid and complete mixing will adequately reduce the effluent's toxicity.

In suitable locations this approach will prevent affects on the major part of a watercourse and its organisms. Another reason for allowing such an area could be as an interim measure while control procedures are being developed (OECD, 1987). The dimensions of a mixing zone should be defined in terms of space, duration and toxicity of the toxic effluent's plume. Allowing a mixing zone should be conditioned on the absence of toxic effects as defined by the NOEC or TUe outside the mixing zone's boundary (Fetterolt, 1973). In some instances, a mixing zone may be divided into two regions. In the immediate area of discharge, acute toxicity (>maximum allowable acute criterium) might be permitted.

In the remainder of the mixing zone and in accordance with time or toxicity limits, chronic toxicity (> maximum allowable chronic criterium) is permitted. Outside this area the usual toxicity criteria for receiving water should apply (NOEC or TUe). The EPA specifies that acute toxicity should be prevented within the mixing zone (US EPA, 1991a).

Important considerations in determining the size of a mixing zone are the volume of dilution water available, and the speed and uniformity of dilution (dilution is most effective when using a high-velocity diffuser). In the US most States specify that the zone should not be as wide as the stream in order to allow a zone of passage for fish. In very few instances the allowable length is given. The size of a zone is determined on a case by case basis taking into account the critical resource areas that need to be protected (US EPA, 1991 a). It is recommended that a mixing zone should be limited to a small area of receiving water located away from valuable fisheries or other sensitive water uses.

The US recommends that mixing zones should be evaluated and used for regulation in cases where complete mixing does not occur within a short distance of the outfall (discharges into large rivers, lakes, estuaries) (US EPA, 1985b). If mixing is assumed to be rapid and complete when it is not, a toxic discharge that appears to meet criteria may cause zones of chronic toxicity that can extend for kilometres.

A.3.1.4. Collection of receiving water to be as dilution water

If the objectives of the test require the use of uncontaminated surface water as dilution water, and the receiving water is uncontaminated, it may be possible to collect a sample of the receiving water close to the outfall, but upstream from or beyond the influence of the effluent. However, if the receiving water is contaminated, it may be necessary to collect the sample in an area "remote" from the discharge site,

matching as closely as possible the physical and chemical characteristics of the receiving water near the outfall.

In the case of freshwaters, the regulatory authority may require that the hardness of the dilution water be comparable to the receiving water at the discharge site. This requirement can be satisfied by collecting uncontaminated surface water with a suitable hardness, or adjusting the hardness of an otherwise suitable surface water by addition of reagents.

Receiving water containing debris or indigenous organisms that may be confused with or attack the test organisms should be filtered through a sieve having 60 µm mesh openings prior to use

A.3.2. Sample complexity and variability

Wastewater may contain a variety of known and unknown substances. In the European chemical Substances Information System (ESIS) the European Inventory of Existing Commercial chemical Substances (EINECS) inventory of existing chemicals list about 100 000 chemicals presumed to be on the European market (http://esis.jrc.ec.europa.eu/). Many of these chemicals are potential wastewater components from manifold sources. In view of the precautionary principle it would be optimal to analyse all substances in a discharge, to determine their concentrations, and to have knowledge of their effects on the environment.

On the basis of such data, efficient measures could be taken to minimise harmful effects. In most cases, however, knowledge of wastewater constituents is very limited. In chemical processes, not only the target substances and products, but also an additional large number of unknown by-products may be synthesised. Moreover, new substances may be formed during biotic or abiotic degradation in the treatment plants. It would require a huge expense to analyse every single substance, if it were possible at all. For most substances, there are not even standardised analytical methods. Information about biological effects of chemicals potentially present in treated effluents would in most cases be unavailable, even if these chemicals were identified and their concentrations were known. According to Matthiessen *et al*., 1993 and Johnston *et al*., 1996 this applies in particular to synergistic or antagonistic effects.

It is not feasible to develop guidelines for all chemicals either because there is insufficient toxicological studies available or because the chemical is currently not available in South Africa or not considered a risk here. There could also be situations where an effluent contains a range of chemicals and complexes, and therefore the chemical make-up might not be well understood. In this instance the complex chemistry might increase or reduce the toxicity of the overall mixture to an unknown degree and so guidelines would be irrelevant. A third possible situation relating to the protection of aquatic ecosystems is where there is a well-founded suspicion that a particular natural community may have atypical sensitivity to one or more contaminants.

Ecological bioassays have the advantage that toxic effects of bioavailable substances on aquatic organisms are measured directly and therefore all kinds of hazardous substances including their degradation products are considered. The results may indicate levels of toxicity and potential environmental impacts without necessarily correlating with chemical group parameters. Ecological bioassays are therefore a useful tool that can be used in these circumstances, although they are mainly used to assess the toxicity of complex effluents and to derive guidelines for the amount of dilution required to safely discharge an effluent to aquatic environments. Ecological bioassays can also be used as a monitoring tool, testing the ambient waters after they have received effluent discharges. The main advantage with using ecological bioassays is that it is not necessary to know the exact chemical make-up of the test effluent and the interactions between the components, to determine potential effects and impacts.

It is therefore evident that effluent monitoring, with regards to group parameters like AOX, TOC and BOD, in combination with bioassays, is able to achieve both a reduction in chemical loading and a decrease of ecotoxicological effects from wastewater. Group parameters like AOX, TOC, BOD provide valuable

information about the efficiency of wastewater treatments and can basically characterise effluents from different industries. Nevertheless, specific chemical characterisation of single substances may still be required and information on the persistence and bioaccumulation of hazardous substances in effluents should not be ignored.

Effluent variability is caused by changes in composition. Studies conducted by the EPA (US EPA, 1991a) showed that the toxicity of effluents vary, and that any one effluent can exhibit significantly varying toxicity to different test organisms over time. The variability can be handled by proper sampling and testing procedures. Effluents were found to be rarely toxic below 10 percent concentrations and not toxic below 0,1 percent concentrations.

If water quality guidelines do not exist for a specific chemical, or if effluents contain a complex range of chemicals, expert advice should be sought from the relevant authorities on whether a current guideline exists or how a guideline might be derived.

Sample types

The decision on whether to collect a grab or composite sample is based on the requirements of the WUL, the objectives of the test, and an understanding of the short and long-term operations and schedules of the discharger. If the effluent quality varies considerably with time, which can occur where holding times within the treatment facility are short, grab samples may seem preferable because of the ease of collection and the potential of observing peaks (spikes) in toxicity. However, the sampling duration of a grab sample is so short that full characterization of an effluent over a 24-h period would require a prohibitive number of separate samples and tests. Collection of a 24-h composite sample, however, may dilute toxicity spikes, and average the quality of the effluent over the sampling period. Sampling recommendations are provided below.

Grab samples collected during peaks of toxicity provide a measure of maximum toxicity if the toxicity of the effluent is highly variable. A grab sample will only reveal the toxicity peak in an effluent if the sample has been collected at the time of the toxicity peak. Grab samples may be necessary if there is little mixing of effluent with the receiving water (US EPA, 1991a,b,c).

A 24 h composite sample may catch the toxicity peaks, but the compositing process may tend to dilute the toxicity resulting in misleading measures of the maximum toxicity of the effluent. Composite samples are recommended for chronic tests where peak toxicity of short duration is of lesser concern (US EPA, 1991a,b,c).

A.3.2.1. Grab samples

Advantages:

- Easy to collect; require a minimum of equipment and on-site time.
- Provide a measure of instantaneous toxicity. Toxicity spikes are not masked by dilution.

Disadvantages:

- Samples are collected over a very short period of time and on a relatively infrequent basis.
- The chances of detecting a spike in toxicity would depend on the frequency of sampling, and the probability of missing spikes is high.

A.3.2.2. Composite samples

Advantages:

- A single effluent sample is collected over a 24-h period.
- The sample is collected over a much longer period of time than grab samples and contains all toxicity spikes.

Disadvantages:

- Sampling equipment is more sophisticated and expensive, and must be placed on-site for at least 24 h.
- Toxicity spikes may not be detected because they are masked by dilution with less toxic wastes.

A.3.3. Sampling equipment

A.3.3.1. Sample containers

The following guidelines should be considered when selection sample containers:

The sample container should be made from chemically inert material, easy to clean and resistant to heating and freezing. Glassware, polyethene or polytetrafluoroethene (PTFE) vessels are recommended.

Samples may be shipped in one or more new plastic bottles. All sample containers should be rinsed with source water before being filled with sample. After use with receiving water or effluents sample containers are punctured to prevent reuse.

A.3.4. Sampling procedures

The choice of representative sampling points, frequency of sampling, etc. is dependent on the objective of the study. Sampling points and method (grab or composite) should be the same as that specified in the client's WUL or recommended by the regulatory agency. The choice of bioassay test should also be outlined in the WUL. This type of permit information should be provided to the relevant laboratory to ensure it may be helpful to supply us with a copy of the permit to ensure proper testing.

Sufficient sample must be collected to perform the required toxicity and chemical tests. Samples should be collected in clean, new, glass/plastic bottles and must be cooled immediately to 4^{o}C. Samples should also be cooled during compositing if at all possible. To minimize the loss of toxicity due to volatilization of toxic constituents, all sample containers should be "completely" filled, leaving no air space between the contents and the lid. A 4 litre sample will provide sufficient sample volume for a battery of four bioassays and chemical analysis.

All sample bottles should be labelled using waterproof ink and include: sample ID, date and time sampled, company name, name of sampler, and whether a grab or composite sample. Chain of Custody forms should be completed, signed by the sampler and submitted with the samples. Any deviations from the procedures given should be documented and described in the data report.

Samples should be packed in coolers with ice or ice blocks and transported to the laboratory as soon as possible. Testing should begin within 36 hours of sample collection. Whilst there is no "holding time" for hazardous waste samples, it is recommended that testing takes place within two weeks of sample collection.

The ISO 5667-16: Water quality -- Sampling -- Part 16: Guidance on bio-testing of samples manual can be consulted as guidance for developing sampling procedures: This manual contains information regarding sampling procedures as well as preservation and pre-treatment of samples in detail.

A.3.5. Sample handling

A.3.5.1.Temperature

- Grab samples should be chilled immediately following collection.
- Composite samples should be chilled as they are collected.

Unless the samples are used in an on-site toxicity test the day of collection (or hand delivered to the testing laboratory for use on the day of collection), it is recommended that they be held at 0-6°C until used to inhibit microbial degradation, chemical transformations, and loss of highly volatile toxic substances.

A.3.5.2. Holding time

Sample holding time begins when the last grab sample in a series is taken (i.e., when a series of four grab samples are taken over a 24-h period), or when a 24 hour composite sampling period is completed. If the data from the samples are to be acceptable for use, the lapsed time (holding time) from sample collection to first use of each grab or composite sample must not exceed 36 h. US EPA, 2002 believes that 36 h is adequate time to deliver the samples to the laboratories performing the tests in most cases.

In the isolated cases, where it can be proved that this delivery time cannot be met, the permitting authority can allow an option for on-site testing or a variance for an extension of shipped sample holding time. The request for a variance in sample holding time should include supportive data which show that the toxicity of the effluent sample is not reduced (e.g., because of volatilization and/or sorption of toxics on the sample container surfaces) by extending the holding time beyond more than 36 h. However, in no case should more than 72 h elapse between collection and first use of the sample. The persistence of the toxicity of an effluent prior to its use in a toxicity test is of interest in assessing the validity of toxicity test data, and in determining the possible effects of allowing an extension of the holding time. Where a variance in holding time (>36 h, but ≤72 h) is requested by a licensee, information on the effects of the extension in holding time on the toxicity of the samples must be obtained by comparing the results of multi-concentration acute toxicity tests performed on effluent samples held 36 h with toxicity test results using the same samples after they were held for the requested, longer period. The portion of the sample set aside for the second test must be held under the same conditions as during shipment and holding.

A.3.5.3. Chlorination

If the effluent has been chlorinated, total residual chlorine must be measured immediately following sample collection.

A.3.6. Sample shipment

Samples collected for off-site toxicity testing are to be chilled to 4±2°C during or immediately after collection, and shipped iced to the performing laboratory. Sufficient ice should be placed with the sample in the shipping container to ensure that ice will still be present when the sample arrives at the laboratory and is unpacked. Insulating material should not be placed between the ice and the sample in the shipping container unless required to prevent breakage of glass sample containers.

A.3.7. Sample receipt

Upon arrival at the laboratory, samples are logged in and the temperature is measured and recorded. If the samples are not immediately prepared for testing, they are stored at 4±2°C until used. Every effort must be made to initiate the test with an effluent sample on the day of arrival in the laboratory, and the sample holding time should not exceed 36 h before first use unless a variance has been granted by the permitting authority.

A.3.8. Sample storage

Sample holding times and temperatures must conform to conditions described in the relevant methods.

When cooled to between 4±2°C and stored in the dark, most samples are normally stable for up to 24 hours. Deep freezing below -18°C in general increases the stability in preservation. In general, biotests are carried out with the original sample.

A.3.9. Sampling Documents

The collection and handling of samples are reviewed to verify that the sampling and handling procedures followed. Chain-of-custody forms are reviewed to verify that samples were tested within allowable sample holding times.

Effluent Samples
Sampling point (including latitude and longitude)
Sample collection method
Collection dates and times
Mean daily discharge on sample collection date
Lapsed time from sample collection to delivery
Sample temperature when received at the laboratory
Physical and chemical data

Receiving Water Samples
Sampling point (including latitude and longitude)
Sample collection method
Collection dates and times
Streamflow (at time of sampling)
Lapsed time from sample collection to delivery
Sample temperature when received at the laboratory
Physical and chemical data

Dilution Water Samples
Source
Collection date(s) and time(s) (where applicable)
Pre-treatment
Physical and chemical characteristics (pH, hardness, salinity, etc.)
Continuous Discharges

If the facility discharge is continuous, but the calculated retention time of the continuously discharged effluent is less than 14 days and the variability of the effluent toxicity is unknown, at a minimum, four grab samples or four composite samples are collected over a 24-h period. For example, a grab sample is taken every 6 h (total of four samples) and each sample is used for a separate toxicity test, or four successive 6-h composite samples are taken and each is used in a separate test.

If the calculated retention time of a continuously discharged effluent is greater than 14 days, or if it can be demonstrated that the wastewater does not vary more than 10% in toxicity over a 24-h period, regardless of retention time, a single grab sample is collected for a single toxicity test.

The retention time of the effluent in the wastewater treatment facility may be estimated from calculations based on the volume of the retention basin and rate of wastewater inflow. However, the calculated retention time may be much greater than the actual time because of short-circuiting in the holding basin. Where short-circuiting is suspected, or sedimentation may have reduced holding basin capacity, a more accurate estimate of the retention time can be obtained by carrying out a dye study.
Intermittent Discharges
If the facility discharge is intermittent, a grab sample is collected midway during each discharge period. Examples of intermittent discharges are:

When the effluent is continuously discharged during a single 8-h work shift (one sample is collected), or two successive 8-h work shifts (two samples are collected).
When the facility retains the wastewater during an 8-h work shift, and then treats and releases the wastewater as a batch discharge (one sample is collected).
When the facility discharges wastewater to an estuary only during an outgoing tide, usually during the 4 hour following slack high tide (one sample is collected).
At the end of a shift, clean-up activities may result in the discharge of a slug of toxic waste, which may require sampling and testing.

A.3.10. Sample pre-treatment

Any sample pre-treatment will alter the laboratory observed results from those observed in the field. All separation methods, however, involve the risk that active components, bound to the particulates, are removed prior to the tests. These pre-treatment approaches therefore need to be documented and incorporated into the interpretation of results.

Wastewater samples can contain large amounts of particulate matter, sludge and sediment which may interfere with the behavioural requirements of the test organisms or with the detection devices (e.g. by photometry). Additionally, wastewater organisms may interfere with the test system (e.g. bacteria with respiration inhibition, protozoa with alga growth) should be removed.

Samples with particulate material may be filtered through 0.45µm in order to assess the dissolved fraction and remove organisms which will interfere with the results. Some test methods (e.g. the *Vibrio fischeri* assay) offer the possibility of determining a correction factor for parameters such as turbidity.

Samples with extreme pH values exceeding the tolerance limits of the test organisms can neutralised. Neutralisation of samples is proposed e.g. in the German test guidelines for ecotoxicity testing of wastewater. Especially when testing for genotoxicity, effluent as well as surface water samples are often highly concentrated. Neutralisation should be omitted if the effect of pH is to be reflected or if pH adjustment is found to cause physical or chemical reactions (e.g. precipitation).

APPENDIX B: PUBLISHED STANDARDS

B.1. US EPA

Whole effluent toxicity tests employ the use of standardized, surrogate freshwater vertebrates, invertebrates, and plants. The EPA has published extensive written protocols listing numerous organisms for toxicity testing (US EPA, 1991b,c). The following are examples of freshwater fish and invertebrates recommended by the EPA for acute lethal toxicity determination of effluents (US EPA, 1985a):

Cold water fish: Rainbow trout *(O. mykiss)*
Warm water fish: Fathead minnow *(Pimephales promelas)*
Cold water invertebrates: Cladocera *(D. magna/pulex, Ceriodaphnia* spp.)

Traditionally, chronic tests are either full life-cycle tests or a shortened test of about 30 days, known as an early life stage test. However, the duration of most of the EPA chronic toxicity tests has been shortened to 7 days by focusing on the most sensitive life-cycle stages. These tests are therefore called short-term chronic tests (Table B.1).

Table B.1: Freshwater tests

Test organism – Trophic level	Method reference/number	Title
Invertebrates	Test Method 2002.0	*Ceriodaphnia dubia* (Cladoceran) acute toxicity test with effluents and receiving waters.
	Test Method 2007.0	Summary of test conditions and test acceptability criteria for mysid, *Mysidopsis bahia*, acute toxicity tests with effluents and receiving waters.
	Test Method 2021.0	*Daphnia pulex* and *Daphnia magna* acute toxicity test with effluents and receiving waters.
Vertebrates	Test Method 2000.0	Fathead minnow, *Pimephales promelas*, acute toxicity tests with effluents and receiving waters.
	Test Method 2004.0	Sheepshead minnow, *Cyprinodon variegatus*, acute toxicity tests with effluents and receiving waters.
	Test Method 2006.0	Silverside, *Menidia beryllina, M. menidia*, and *M. peninsulae*, acute Toxicity tests with effluents and receiving waters.
	Test Method 2019.0	Rainbow trout, *Oncorhynchus mykiss*, and brook trout, *Salvelinus fontinalis*, acute toxicity tests with effluents and receiving waters.
	OPPTS 850.1075 (1996)	Fish Acute Toxicity Test, Freshwater and Marine.
Algae	Test Method 1003.0	Green alga, *Selenastrum capricornutum*, growth.
Invertebrates	Test Method 1002.0	Daphnia, *Ceriodaphnia dubia*, survival and reproduction tests with effluents and receiving waters.

According to Slabbert et al 1998 the following tests were in use or under development for non-threshold human health toxicants (assessing carcinogenicity or mutagenicity):

- *Salmonella typhimurium* assay (Ames Test) – endpoint: gene mutation, response measured in revertant colonies/e effluent;
- Escherichia coli SOS assay (SOS Chromotest) – endpoint: DNA damage, response measured as the change in optical density;
- Sister-chromatid exchange assay (SCE) – endpoint: DNA damage, response measured in SCE per chromosome/fi effluent;
- Chinese hamster ovary cell assay – endpoint: gene mutation, response measured as % survival/f effluent;
- Medaka fish tumour assay] – endpoint: tumour formation, response measured in frequency of tumours at a given site/effluent concentration.

B.2. ISO

The following ISO standard methods are available for water quality testing:

Table B.2: Freshwater tests

Test organism	Method reference/number	Title
Invertebrates	*ISO 6341:2012**	*Water quality -- Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea) -- Acute toxicity test*
	BS EN ISO 6341:2012 Revises BS EN ISO 6341:1996 (British Standard)	*Water quality. Determination of the inhibition of the mobility of Daphnia magna Straus (Cladocera, Crustacea). Acute toxicity test*
	ISO 14380:2011#	*Water quality -- Determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca)*
	ISO 11268-1:2012 Revises ISO 11268-1:1993	Soil quality – Effects of pollutants on earthworms – Part 1: Determination of acute toxicity to Eisenia fetida/Eisenia andrei
Vertebrates	ISO 15088:2007	Water quality -- Determination of the acute toxicity of wastewater to zebrafish eggs (*Danio rerio*)
	ONORM EN ISO 7346-1:1998 (Austrian Standard)	*Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish (Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] – Part ....(abbreviated)*
	ISO 7346-1:1996#	*Water quality -- Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] -- Part 1: Static method*
	DIN EN ISO 7346-1:1998 (ISO 7346-1:1996); German version EN ISO 7346-1:1997 (Foreign Standard)	*Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)] – Part 1: Static method*
Bacteria	*ISO 11348-3:2007#*	*Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria*
	ISO 10712:1995	Water quality -- *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test)
Algae	*ISO 8692:2012**	*Water quality -- Fresh water algal growth inhibition test with unicellular green algae*
	DIN 38412-33:1991	German standard methods for the examination of water, wastewater and sludge; bio-assays (group L); determining the tolerance of green algae to the toxicity of wastewater (Scenedesmus chlorophyll fluorescence test) by way of dilution series (L 33) (Foreign Standard)
	ISO 14442:2006	Water quality -- Guidelines for algal growth inhibition tests with poorly soluble materials, volatile compounds, metals and wastewater
	ISO/TR 11044:2008	Water quality -- Scientific and technical aspects of batch algae growth inhibition tests
Plants	ISO 20079:2005	Water quality -- Determination of the toxic effect of water constituents and wastewater on duckweed (*Lemna minor*) -- Duckweed growth inhibition test
	ISO 22030:2005	Soil quality – Biological methods – Chronic toxicity in higher plants
	DIN EN ISO 22030:2011 (ISO 22030:2005);	Soil quality – Biological methods – Chronic toxicity in higher plants

	German version EN ISO 22030:2011 (Foreign Standard)	
	ONORM EN ISO 22030:2011 (ISO 22030:2005) (Austrian Standard)	Soil quality – Biological methods –- Chronic toxicity in higher plants
	SS-EN ISO 22030:2011 (ISO 22030:2005) (Swedish Standard)	Soil quality – Biological methods – Chronic toxicity in higher plants
Invertebrates	*ISO 20665:2008#*	*Water quality -- Determination of chronic toxicity to Ceriodaphnia dubia*
	ISO 10706:2000	Water quality -- Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea)
	ISO 20666:2008	Water quality -- Determination of the chronic toxicity to *Brachionus calyciflorus* in 48 h
Vertebrates	ISO 10229:1994	Water quality -- Determination of the prolonged toxicity of substances to freshwater fish -- Method for evaluating the effects of substances on the growth rate of rainbow trout (*Oncorhynchus mykiss* Walbaum (Teleostei, Salmonidae))
	ISO 15088:2007	Water quality – Determination of the acute toxicity of wastewater to zebrafish eggs (Danio rerio)

\# *Standards adopted by the South African Bureau of Standards (SABS).*
* *Standards in the process of adoption by the South African Bureau of Standards (SABS).*

Table B.3: Sediment and soils tests

Test organism	Method reference/number	Title
Bacteria	ISO 21338:2010	Water quality -- Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri* (kinetic luminescent bacteria test)
Algae		
Plants	ISO 16191:2013	Water quality -- Determination of the toxic effect of sediment on the growth behaviour of *Myriophyllum aquaticum*
Invertebrates	ISO 14371:2012	Water quality -- Determination of fresh water sediment toxicity *to Heterocypris incongruens* (Crustacea, Ostracoda)
	ISO 10872:2010	Water quality -- Determination of the toxic effect of sediment and soil samples on growth, fertility and reproduction *of Caenorhabditis elegans* (Nematoda)
	ISO 16303:2013	Water quality -- Determination of toxicity of fresh water sediments using *Hyalella* Azteca
	DIN 38412-30:1989 German (FOREIGN STANDARD)	standard methods for the examiniation of water, wastewater and sludge; bio-assays (group L); determining the tolerance of Daphnia to the toxicity of wastewater by way of a dilution series (L 30)
	DIN EN ISO 15952:2011 S (ISO 15952:2006); German version EN ISO 15952:2011 (Foreign Standard)	Soil quality – Effects of pollutants on juvenile land snails (Helicidae) – Determination of the effects on growth by soil contamination
	ISO 20963:2005	Soil quality – Effects of pollutants on insect larvae (*Oxythyrea funesta*) – Determination of acute toxicity
	BS EN ISO 20963:2011	Soil quality. Effects of pollutants on insect larvae (*Oxythyrea funesta*). Determination of acute toxicity (British Standard)

\# *Standards adopted by the South African Bureau of Standards (SABS).*
* *Standards in the process of adoption by the South African Bureau of Standards (SABS).*

Table B.4: Human health tests

	Method reference/number	Title
Genotoxicity	ISO 11350:2012	Water quality – Determination of the genotoxicity of water and wastewater – *Salmonella* / microsome fluctuation test (Ames fluctuation test)
	ISO 13829:2000	Water quality – Determination of the genotoxicity of water and wastewater using the UMU-test
	ISO 16240:2005	Water quality – Determination of the genotoxicity of water and wastewater – *Salmonella* / microsome test (Ames test)
	ISO 21427-1:2006	Water quality – Evaluation of genotoxicity by measurement of the induction of micronuclei – Part 1: Evaluation of genotoxicity using amphibian larvae
	ISO 21427-2:2006	Water quality – Evaluation of genotoxicity by measurement of the induction of micronuclei – Part 2: Mixed population method using the cell line V79

B.3. OECD

The following tests, applicable in the water field, are recommended in "OECD Guidelines for Testing of Chemicals" (1981):

Table B.5: Acute and chronic test

Test organism	Method reference/number	Title
Acute toxicity test		
Invertebrates	202: 2004	Daphnia sp. Acute Immobilisation Test
	207: 1984	Earthworm, Acute Toxicity Tests
	235: 2011	Chironomus sp., Acute Immobilisation Test
Vertebrates	203: 1992	Fish, Acute Toxicity Test
	236: 2013	Fish Embryo Acute Toxicity (FET) Test
Chronic toxicity tests		
Bacteria	224: 2007	Determination of the Inhibition of the Activity of Anaerobic Bacteria
Algae	201: 2011	Freshwater Alga and Cyanobacteria, Growth Inhibition Test
Plants	221: 2006	Lemna sp. Growth Inhibition Test
Invertebrates	211: 2008	Daphnia magna Reproduction Test
	211: 2012	Daphnia magna Reproduction Test
	220: 2004	Enchytraeid Reproduction Test
	222: 2004	Earthworm Reproduction Test (Eisenia fetida/Eisenia andrei)
	232: 2009	Collembolan Reproduction Test in Soil
Vertebrates	204: 1984	Fish, Prolonged Toxicity Test: 14-Day Study
	210: 1992	Fish, Early-Life Stage Toxicity Test
	210: 2013	Fish, Early-life Stage Toxicity Test
	212: 1998	Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages
	215: 2000	Fish, Juvenile Growth Test
	229: 2010	Fish Short Term Reproduction Assay
	229: 2012	Fish Short Term Reproduction Assay
	230: 2009	21-day Fish Assay
	231: 2009	Amphibian Metamorphosis Assay
	234: 2011	Fish Sexual Development Test

Table B.6: Sediment and soil test

Test organism	Method reference/number	Title
Plants	238: 2014	Sediment-Free *Myriophyllum Spicatum* Toxicity Test
	239: 2014	Water-Sediment *Myriophyllum Spicatum* Toxicity Test
	227: 2006	Terrestrial Plant Test: Vegetative Vigour Test
	208: 2006	Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
Invertebrates	218: 2004	Sediment-Water Chironomid Toxicity Using Spiked Sediment
	219: 2004	Sediment-Water Chironomid Toxicity Using Spiked Water
	233: 2010	Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment
	225: 2007	Sediment-Water Lumbriculus Toxicity Test Using Spiked Sediment
Vertebrates		

The OECD guidelines also include a list of tests for human health effect assessment of chemicals. Some of the recommended genetic toxicology tests which are applied in the water field are: the *S. typhimurium* assay; the *E. coli* reverse mutation assay; and the in vitro sister chromatid exchange assay using mammalian cells.

B.4. Environment Canada

A wide range of biological tests are conducted in the Canadian Federal Environmental Protection Laboratories. Generally, the tests employed measure both acute and chronic toxicity with their corresponding lethal and sublethal effects and include several representative species of different trophic levels (Table 2) (MacGregor and Wells, 1984; Sergy, 1987). The amount, manner and effectiveness of use of the tests are not the same in the different laboratories. This is because of different federal regional strategies, mandates, expertise and budgets (Blaise *et al.,* 198B). The types of tests are as consistent as possible with OECD guidelines, US EPA and ASTM methods. The application of the biological tests in environmental protection activities includes four major steps: problem identification; problem assessment; control or intervention; and control evaluation (Sergy, 1987). The application of bioassays for drinking water protection in Canada is limited to research institutions like the National Water Research Institute, Canada Centre for Inland Waters, Burlington. The battery approach is followed using a range of tests, including several microbial tests (Dutka and Kwan, 1981; 1988).

Table B.7: Freshwater tests

Test organism	Method reference/number	Title
Acute tests		
Invertebrates	En49-24/1-11E-PDF: July 1990, with May 1996 amendments	Biological Test Method – Acute Lethality Test Using *Daphnia* spp.
	En49-24/1-14E-PDF: EPS 1/RM/14, December 2000	Biological Test Method – Reference Method for Determining Acute Lethality of Effluents to *Daphnia magna*
Vertebrates	En49-24/1-9E-PDF: EPS 1/RM/9, July 1990, with May 1996 and May 2007 amendments	Biological Test Method – Acute Lethality Test Using Rainbow Trout
	En49-24/1-13E-PDF: EPS 1/RM/13, c2000, with May 2007 amendments	Biological Test Method – Reference Method for Determining Acute Lethality of Effluents to Rainbow Trout
	EPS 1/RM/50, 2008	Procedure for pH Stabilization During the Testing of Acute Lethality of Wastewater Effluent to Rainbow Trout
	En84-103/2008E-PDF, 2008	Supplementary Background and Guidance for Investigating Acute Lethality of Wastewater Effluent to Rainbow Trout
Short term chronic tests		
Bacteria	EPS1/RM/24, 1992	Biological Test Method: Toxicity Test Using Luminescent Bacteria
Algae	En49-7/1-25E-PDF: EPS 1/RM/25, March 2007	Biological Test Method: Growth Inhibition Test Using a Freshwater Alga
Plants		
Invertebrates	En49-7/1-21E-PDF: EPS 1/RM/21, February 2007	Biological Test Method – Test of Reproduction and Survival Using the Cladoceran *Ceriodaphnia dubia*
	EPS1/RM/41, 2001	Biological Test Method: Test for Survival and Growth in Sediment Using Spionid Polychaete Worms (*Polydora cornuta*)
Vertebrates	En49-7/1-22E, 2011	Biological Test Method: Test of Larval Growth and Survival Using Fathead Minnows EPS 1/RM/22
	EPS 1/RM/28, 1998	Biological Test Method: Toxicity Tests Using Early Life Stages of Salmonid Fish (Rainbow Trout)

Table B.8: Sediment test

Test organism – Trophic level	Method reference/number	Title
Short term chronic tests		
Bacteria	EPS 1/RM/42, 2002	Biological Test Method: Reference Method for Determining the Toxicity of Sediment Using Luminescent Bacteria in a Solid-Phase Test
Plants	EPS 1/RM/37, 2007	Biological Test Method: Test for Measuring the Inhibition of Growth Using the Freshwater Macrophyte, *Lemna minor*
	En49-7/1-45E-PDF: EPS 1/RM/45, February 2005 (with June 2007 amendments)	Biological Test Method – Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil
	En49-7/1-56E, 2013	Biological Test Method: Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region
Invertebrates	En49-24/1-32E-PDF: EPS 1/RM/32, December 1997	Biological Test Method. Test for Survival and Growth in Sediment Using Larvae of Freshwater Midges (*Chironomus tentans* or *Chironomus riparius*)
	En49-7/1-33E, 2012	Biological Test Method: Test for Survival and Growth in Sediment and Water Using the Freshwater Amphipod Hyalella azteca
	En49-7/1-43E-PDF: EPS 1/RM/43, June 2004	Biological Test Method. Tests for Toxicity of Contaminated Soil to Earthworms (Eisenia andrei, Eisenia fetida, or Lumbricus terrestris)
	En49-7/1-47E-PDF: EPS 1/RM/47, September 2007	Biological Test Method. Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil

B.5. ASTM

The following ASTM standard methods developed for biological effect and environmental fate testing can be used for water testing:

- Acute toxicity tests on aqueous effluents with fish, macro-invertebrates and amphibians {ASTM, 1988a,b)
- Early life-stage toxicity tests with fish (ASTM, 1988c)
- Static acute toxicity tests on wastewaters with D. magna (ASTM, 1984)
- Renewal life-cycle toxicity tests with D, magna (ASTM, 1987a)
- Renewal toxicity tests with C. dubia (ASTM, 1989)
- Algal growth potential testing with S. capricornutum (ASTM, 1987b)

Table B.9: Freshwater tests

Test organism – Trophic level	Method reference/number	Title
Acute tests		
Invertebrates	ASTM E1440-91(2012) Revises ASTM E1440-91(2004)	Standard Guide for Acute Toxicity Test with the Rotifer *Brachionus* sp.
Vertebrates	ASTM E1192-97(2008) Revises ASTM E1192-97(2003)	Standard Guide for Conducting Acute Toxicity Tests on Aqueous Ambient Samples and Effluents with Fishes, Macroinvertebrates, and Amphibians
Short term chronic tests		
Algae	ASTM E1218-04(2012) Revises ASTM E1218-04e1	Standard Guide for Conducting Static Toxicity Tests with Microalgae
Invertebrates	ASTM E1193-97(2012) Revises ASTM E1193-97(2004)	Standard Guide for Conducting Daphnia magna Life-Cycle Toxicity Tests
	ASTM E2455-06(2013) Revises ASTM E2455-06	Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels
	ASTM E1562-00(2013) Revises ASTM E1562-00(2006)	Standard Guide for Conducting Acute, Chronic, and Life-Cycle Aquatic Toxicity Tests with Polychaetous Annelids
Vertebrates	ASTM E1439-12 Revises ASTM E1439-98(2004)	Standard Guide for Conducting the Frog Embryo Teratogenesis Assay-Xenopus (FETAX)
Other	ASTM E1604-12 Revises ASTM E1604-94(2007)	Standard Guide for Behavioral Testing in Aquatic Toxicology
	ASTM E1711-12 Revises ASTM E1711-95(2008)	Standard Guide for Measurement of Behavior During Fish Toxicity Tests

B.10: Sediment

Test organism – Trophic level	Method reference/number	Title
Acute tests		
Invertebrates	ASTM E1706-05(2010)	Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates
	ASTM E1611-00(2013)	Standard Guide for Conducting Sediment Toxicity Tests with Polychaetous Annelids
	ASTM E2172-01(2008) Revises ASTM E2172-01	Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode Caenorhabditis elegans
	ASTM E1676-12 Revises ASTM E1676-04	Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm *Eisenia fetida* and the Enchytraeid Potworm *Enchytraeus albidus*
Vertebrates	ASTM E2591-07(2013) Revises ASTM E2591-07	Standard Guide for Conducting Whole Sediment Toxicity Tests with Amphibians
Other	ASTM E1850-04(2012) Revises ASTM E1850-04	Standard Guide for Selection of Resident Species as Test Organisms for Aquatic and Sediment Toxicity Tests
Short term chronic tests		
Plants	ASTM E1963-09 Revises ASTM E1963-02	Standard Guide for Conducting Terrestrial Plant Toxicity Tests
Vertebrates	ASTM E1241-05(2013) Revises ASTM E1241-05	Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes
Other		
	ASTM E1525-02(2008) Revises ASTM E1525-02	Standard Guide for Designing Biological Tests with Sediments

APPENDIX C: WULA ASSESSMENT CHECKLIST

WULA ASSESSMENT CHECKLIST: Department of Water Affairs

NO.	DOCUMENT	Provided by	YES	NO	N/A
1	Fully and correctly completed license application forms	Applicant			
2	Supplementary Forms	Applicant			
3	Copy receipt of Registration fee of R114.00	Applicant			
4	Certified ID of applicant/company registration certificate	Applicant			
5	Copy of property's title deed	Applicant			
6	Copy of property's zoning document	Applicant			
7	A letter of consent from the registered land owner if property is leased	Applicant			
8	A copy of 1:50 000 topographic map / 1:10 000 indicating map name number of farm boundaries including subdivision.	Applicant			
9	Legible map with a colour coded legend showing the water uses in relation to the affected watercourse.	Applicant			
10	Technical reports	Applicant			
	• Environmental Impact Assessment Report/Basic Assessment Report	Applicant			
	• Environmental Management Plan	Applicant			
	• Stormwater management plan	Applicant			
	• Geotechnical Site Investigation	Applicant			
	• Impact assessment associated with the characteristics(Flow Regime, Water quality, Biota, Riparian and riparian habitat) of a watercourses/wetland	Applicant			
	• Wetland Delineation and assessment Report including PES and EIS	Applicant			
	• Wetland Rehabilitation and management plan	Applicant			
	• Rehabilitation plan for affected watercourses/rivers	Applicant			
	• Monitoring programme for the affected watercourse	Applicant			
	• Monitoring programme for the affected wetland	Applicant			
	• Civil Designs of the structures that will affect a watercourse/wetland	Applicant			
11	Comprehensive method statement for the activities occurring within the affected watercourse/wetland	Applicant			
12	Master plan indicating temporary and permanent infrastructure, diversions, 1:100 year floodline, no-go areas, demarcated areas, wetlands, sensitive areas, stockpiles, material lay down areas, rest, eat areas, camp site, access, parking, offices, storerooms, housing, etc.	Applicant			
13	Environmental Authorisation from Environmental Affairs (RoD)	Applicant			
14	Proof of Public Participation	Applicant			
15	Proof of BBBEEE status considering to redress the results of past racial and gender discrimination according to Section 27 (1b) of NWA of 1998 No. 36 (BEE Certificate or motivation)	Applicant			
16	Section 27(1) Motivation of National Water Act, 1998	Applicant			
17	Reserve Determination	Department			
18	Reserve Determination Request	Department			

APPENDIX D: HAZARD CLASSIFICATIONS

D.1. CLASSIFICATION SYSTEM FOR INDUVUDUAL TEST RESULTS

In order to provide an ecological hazard assessment there is a need for assessment criteria. These criteria would generally be numerical values with which the test results can be compared to pronounce on the expected impact implied by the test result.

During an investigation by DWS (DWAF, 2003) RIZA provided some assessment criteria for the Dutch TEM method as indicated in Table 1.

Table D.1. Criteria for ecological hazard assessment for discharges provided for the TEM method by RIZA in The Netherlands.

Test result	*Hazard description*
<1 TUa*	Not acutely toxic
1-2 TUa	Negligibly acutely toxic
2-10 TUa	Mildly acutely toxic
10-100 TUa	Acutely toxic
>100 TUa	Highly acutely toxic

* TUa is an acute toxic unit and is derived from calculation in an acute toxicity test. For a discharge the TUa = 100/LC50.

The assessment criteria in Table 1 have been based not only on theoretical considerations but on practical observation. There is therefore cogent reason to accept these. In the “Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach” proposal document, DWAF, 2003 indicated that DWS recognise however that what constitutes an ecological hazard in one country may not constitute an ecological hazard in another country and that we would have to validate these criteria locally before finally adopting them in the DEEEP method. The data gathered in the testing and pilot implementation of this method in South Africa as well as consultation with various role players will help to adapt and refine these criteria.

Principles that need to be considered in refining and adapting assessment criteria would include:
The Department of Water and Sanitation recognises that the ecological effect is not in the discharge, but in the receiving water. Furthermore, we recognise that receiving water systems are currently in different ecological states and may in future be managed with different ecological states in mind that represent different levels of ecological risk and hazard. This supplies added reason to generate receiving water specific criteria in addition to those for General Authorisation.
The assessment of a discharge should not be so lenient as to cause damage to the ecosystem – damage that may be costly or impossible to repair. On the other hand it should not be so strict as to place an unnecessary burden on the discharger worth consequent economic and other implications.

This type of information about criteria could be collected during the testing and pilot implementation of the methodology, but like all other criteria, we believe that the DEEEP assessment criteria should be reviewed from time to time.

D.2. CLASSIFICATION SYSTEM FOR BATTERY TEST RESULTS

In analogy to biotic indexes, various toxicity classification and scoring systems have been worked out for effluents, wastewaters, sediments and dredged materials, and soils, such as the pT (Krebs, 1988), PEEP

(Costan et al.,1993), PAF (Roghair et al., 1997) and SEDTOX (Bombardier and Birmingham, 1999), to quote only a few of the many systems proposed.

For a complete overview of the degree of pollution and toxic hazard of aquatic environments, toxicity scoring systems are combined with chemical analyses and with biotic indices in the so-called TRIAD approach, a concept developed originally by Chapman (1986, 1990) for sediment quality assessment.

Despite their merits, these toxicity classification and scoring systems have their own bottlenecks and limitations which restrict their application to a very small number of well-equipped and highly specialised laboratories.

The “Classification System” developed by Persoone et al, 2003 (as part of the framework of a cooperation agreement between Flanders and Central and Eastern Europe: the so-called Fita4 programme) was selected for the current project due to its simplicity, ease of use and cost cost-effective input required. This method has been successfully applied internationally since 2000 by the participating laboratories on samples of river waters, groundwaters, drinking waters, mine waters, sediment pore waters, industrial effluents, soil leachates and waste dump leachates.

Principals

The “Classification System” developed by Persoone et al, 2003 can be applied to the following two different groups of samples:

The first group is for “gross” determination of the degree of toxic contamination of “natural waters/river samples”, such as the upstream and downstream samples indicated in license conditions. For this group, screening test results would be required as input with on “non-diluted” samples.
The second group is for the “quantification” of the toxicity of wastewater/effluents prior to their release into the aquatic environments. This would typically be the effluent specified in license conditions that is discharged into the aquatic environment. For the wastewaters/effluents, however, bioassays have to be performed in a second step on a dilution series, for which more than 50% effect has been found in the original wastewater.

For both these sample types the “Classification Scoring System” ranks the natural waters/river water or the wastewaters/effluent in 5 classes of increasing hazard/toxicity, with calculation of a “weight score” for the concerned hazard/toxicity class.
The principles of the “Classification Scoring System” can be summarised as follows:

To make a clear distinction between the two systems, the evaluation system for the natural waters/river water should be called the “Hazard Classification System”, and that for wastewater/effluents should be named the “Toxicity Classification System”. Given the need for practicality and low costs, both systems shall be based on a (small) battery of environmental bioassays of short exposure time and with test species representative of different trophic levels. Input from at least three bioassays from different trophic levels should be included in the scoring of samples. The scoring systems will hence only reflect “acute” hazards.
The assays on natural waters shall only be performed on the original sample (i.e. without a dilution series), and the findings should be expressed as percentage effect for each bioassay. For the wastes discharged into the aquatic environment, the tests shall initially be applied to “non-diluted” samples. Assays on a full dilution series should, however, be performed at the next stage with all the bioassay resulting in >50% effect have been found in the original sample, in order to calculate $L(E)C_{50}$ values and the derived Toxic Units.
The scoring systems shall comprise 5 classes ranging from “not acutely hazardous/toxic” to “highly acute hazardous/toxic”.

Weight scores shall be calculated for each hazard/toxicity class to indicate the quantitative importance of the effects in that class.

The test battery which was used initially was composed of the following Toxkit microbiotests (extended by some participants to the bacterial luminescence inhibition assay on *Vibrio fischeri*):

72h algal growth inhibition with *Selenastrum capricornutum* (renamed *Raphidocelis subcapitata* and more recently *Pseudokirchneriella subcapitata* (Algaltoxkit F).
24h ciliate growth inhibition with *Tetrahymena thermophila* (Protoxkit F).
24h rotifer mortality with *Brachionus calyciflorus* (Rotoxkit F).
24h crustacean mortality with *Thamnocephalus platyurus* (Thamnotoxkit F) or alternatively
48h crustacean acute immobilisation with *Daphnia magna* (Daphtoxkit F).

A detailed description of the hazard classification system for natural waters and the toxicity classification system for wastes discharged into the aquatic environment is given below. It may be emphasised that although, for the obvious reasons given above, these classification systems have been developed on the basis of a test battery composed of “culture independent” microbiotests, their principles are also valid and suited for application and/or extension to “conventional” bioassays.

E.2.1. Hazard classification system for natural/river water
The Hazard Classification System principal is summarised as an onestep determination of the acute toxic hazard of natural/river water on non-diluted samples, with a battery of bioassays.

This system is suited for application to all natural/river freshwater such as:

still waters (ponds, lakes, water reservoirs, groundwaters, tap waters) and
running waters (rivers, streams, creeks), and by extension also
to rainwaters,
sediment pore waters and
horizontal soil run-off and soil percolates.

The classification system is based on two values: a ranking in 5 acute hazard classes, and a weight score for each hazard class.

Step 1 Determine Hazard Class (see Table E.1 for summary)
After determination of the percentage effect (hereafter referred to as “EP”) obtained with each of the bioassays, the water is ranked into one of the following 5 classes on the basis of the highest toxic response shown by at least one of the tests applied:

Class I: no acute hazard = none of the tests shows a toxic effect (*i.e. an effect value that is significantly higher than that in the controls)*
Class II: slight acute hazard = a statistically significant EP is reached in at least one test, but the effect level is below 50%
N.B. To determine if the percentage effect observed in the water sample is significantly different from that in the control, one should analyse the data with a statistical programme. Alternatively one can use the 20% effect level as being the lowest EP considered to have a significant toxic impact.
Class III: acute hazard = the EP50 is reached or exceeded in at least one test, but the effect level is below 100%
Class IV: high acute hazard = the EP100 is reached in at least one test
Class V: very high acute hazard = the EP100 is reached in all the tests

Step 2 Determine Weight Scores

A weight score is calculated for each hazard class, to indicate the quantitative importance (weight) of the toxicity in that class. This weight score is expressed in % and will range from 25% (only one test in the

battery has reached the toxicity level of that class) to 93 % (all tests but one have reached the toxicity level of that class). *The rationale for this additional scoring is that the higher the weight score, the more this expresses the toxic hazard of the water in the concerned class.*

Calculation of the weight scores starts with allocation of a test score for the effect results of each bioassays performed in the battery of tests as follows:

No « significant » toxic effect = score 0
Significant toxic effect, but < EP50 = score 1
Toxic effect > EP50, but <EP100 = score 2
EP100 = score 3

This followed by the following calculation:

$$\text{Class weight score} = \frac{\sum \text{all test scores}}{n}$$

(n = number of tests performed)

To determine the class weight score in % the following calculation is done:

$$\text{Class weight score in \%} = \frac{\text{Class score}}{\text{Maximum class weight score}} \times 100$$

N.B. Growth stimulation instead of growth inhibition with the Algaltoxkit points to the potential for eutrophication of the concerned waterbody, and hence to an indirect hazard.

Table D.2. Hazard classification system for natural/river water.

Effect Potential (EP)	Hazard Class		Symbol
<20%	Class I	No acute hazard	☺
20% ≤EP<50%	Class II	Slight acute hazard	☹
50%≤EP<100%	Class III	Acute hazard	☠
EP = 100% in at least one test	Class IV	High acute hazard	☠☠
EP = 100% in all tests	Class V	Very high acute hazard	☠☠☠

E.2.2. Toxicity classification system for wastes discharged into the aquatic environment

The Toxicity Classification System principal is summarised as a two-step determination and quantification of the acute toxicity of the liquid wastes/effluents or leachates with a battery of bioassays.

In the first step the toxicity is determined on non-diluted samples.
In the second step, toxicity tests are performed on a dilution series of the samples, with those bioassays resulting in more than 50% effect in the non-diluted sample.

This system is suited for application to any kind of liquid waste discharged in natural waters, without or after treatment, and by extension also to leachates/percolates from waste dumps and leachates/percolates from contaminated soils.

The classification system is based on two values: a ranking in 5 acute hazard classes, and a weight score for each toxicity class.

Step 1 Determine Hazard Class (see Table E.2 for summary)
The effect results obtained with each microbiotest are transformed into Toxic Units (TU) with the formula: TU = [1/L(E)C50] x 100

The samples are classified into one of the following categories on the basis of the highest number of TU found in one of the tests of the battery.

Class I: no acute toxicity (ideal) = none of the tests shows a toxic effect (i.e. an effect value significantly higher than that in the controls).
Class II: slight acute toxicity (acceptable) = the effect percentage observed in at least one toxicity test is significantly higher than in the control, but is below 50% (< 1 TU).
N.B. Analogous to the scoring system for natural waters, the “20% effect level” can be used as the « lowest » EP considered to have a significant toxic impact. 20% effect corresponds to 0.4 TU (since 50% effect = 1 TU, 20% effect is equivalent to 0.4 TU; 30% effect = 0.6 TU and 40% effect = 0.8 TU).
Class III: acute toxicity (tolerable) = the L(E)C50 is reached or exceeded in at least one test, but in the 10 fold dilution of the sample the effect is lower than 50% (= 1-10 TU)
Class IV: high acute toxicity (unacceptable) = the L(E)C50 is reached in the 10-fold dilution for at least one test, but not in the 100-fold dilution (= 10-100 TU)
Class V: very high acute toxicity (unacceptable) = the L(E)C50 is reached in the 100-fold dilution for at least one test, (= >100 TU)

Step 2 Determine Weight Scores
In analogy to the classification system for natural waters, a weight score is calculated in the same way for each toxicity class, to indicate the quantitative importance (weight) of the toxicity in that class.

Calculation of the weight scores starts with allocation of a test score for the effect

No significant toxic effect	= score 0
Significant toxic effect, but < L(E)C50 (= < 1 TU)	= score 1
1-10 TU	= score 2
10-100 TU	= score 3
>100 TU	= score 4

This followed by the following calculation:

$$\text{Class weight score} = \frac{\sum \text{all test scores}}{n}$$

(n = number of tests performed)

To determine the class weight score in % the following calculation is done:

$$\text{Class weight score in \%} = \frac{\text{Class score}}{\text{Maximum class weight score}} \times 100$$

The threshold values for each hazard/toxicity class and the symbols that were found appropriate to visually quantify the degree of hazard/toxicity for the respective classes, are given in Tables 1 and 2 respectively for the hazard classification system for natural waters and for the toxicity classification system for wastes.

Table D.3. Toxicity classification system for wastes discharged into the aquatic environment.

Toxicity Unit (TU)	TOXICITY CLASS		Symbol
< 0.4	Class I	No acute toxicity (Ideal)	☺
0.4 <TU< 1	Class II	Slight acute toxicity (Acceptable)	☹
1 <TU< 10	Class III	Acute toxicity (Tolerable)	☠
10 <TU< 100	Class IV	High acute toxicity (Unacceptable)	☠☠
TU > 100	Class V	Very high acute toxicity (Unacceptable)	☠☠☠