GUIDANCE
FOR THE SELECTION OF TOXICITY TESTS
IN SUPPORT OF THE INFORMATION REQUIREMENTS
OF THE NATIONAL WATER ACT

Report to the
Water Research Commission

by

JL Slabbert[1] and K Murray[2]
[1]NRE, CSIR Pretoria; [2] Insight Modelling Services CC

WRC Report No 1211/1/10
ISBN 978-1-4312-0088-7

March 2011

DISCLAIMER

This report has been reviewed by the Water Research Commission (WRC) and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the WRC, nor does mention of trade names or commercial products constitute endorsement or recommendation for use

EXECUTIVE SUMMARY

1. Background and Motivation

The National Water Act (NWA) (1998) makes provision for two inter-dependent and complementary strategies of resource-directed measures and source-directed controls to protect water resources. A crucial implication of the NWA is an effect-based approach to resource management (Jooste, 2000). This effect-base is apparent in both the resource-directed measures and the source-directed controls. Within the context of the regulatory use of toxicity assessments in water resource management in South Africa, effect assessments could be applied within a law enforcement context, namely to set standards used in source-directed controls. An effect 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.

Biological toxicity tests are ideally suited to assess the effect of stressors. A large number and variety of toxicity tests are internationally available for aquatic toxicity testing. A range of toxicity tests have also been established for South African use. However, 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.

Our situation in South Africa 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.

2. Objective

The objective of this project was to establish a guide for the selection of toxicity tests that support the information requirements of the National Water Act.

In order to clarify the objective, specific phrases are highlighted:

- ‘guide’ refers to a manual, tool or mechanism;
- ‘for the selection of toxicity tests’ means that this guide is required to assist in choosing appropriate toxicity tests;
- ‘toxicity tests that support the information requirements of the National Water Act’ means that these tests will specifically focus on the information that is required by the National Water Act;
- ‘information requirements of the National Water Act’ refers to phrases, descriptions, objectives in the Act that intimate the need for data, information or decision support from toxicity tests.

3. Methodology

A highly consultative and interactive process was followed during the execution of the project. Both scientists and water quality managers (DWAF) participated in the project. The project was executed in distinct tasks, namely:

- plan project;
- analyse regulatory framework;
- compile inventory of tests;
- integrate information; and
- produce guide.

As part of project planning, a core project team met a number of times with participants from DWAF to discuss the boundaries and requirements of the project, and to confirm the structure for project execution. Concerns about possible subjectivity by project team members in recommending the most suitable toxicity tests for inclusion in the guide were addressed by executing the project in separate tasks. To avoid subjectivity of the ultimate users of the guide it was important to present the ultimate guide in a format that would limit subjectivity. It was agreed that for the regulatory framework, the existence of actual toxicity tests to satisfy the identified requirements was deemed irrelevant. The existence of necessary infrastructure and capacity to perform the tests and to assess and report results were also deemed irrelevant. The focus was on the type of information that toxicity tests can provide in terms of NWA requirements. The advantage of this was that the ultimate guide will identify gaps in current capacity and thus help focus future research and development.

The term 'toxicity test' often has different meanings for scientists and water quality managers. To ensure a common understanding for project execution, the following definition was adopted:

> A **toxicity test**
> 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.

The scope of toxicity testing was further delineated by detailing the capabilities of testing, but also highlighting the features that are excluded from toxicity testing.

At the onset of the task 'Analyse regulatory framework' it was felt that the term 'regulatory' was somewhat misleading, since regulations *per se* comprise only one of the many parts that exist within the NWA. The term 'regulatory' was thus changed to 'water quality management'. It was also decided to use the term 'context' to refer to NWA chapters, parts and sections that can benefit from information from toxicity tests (*e.g.* NWA context, resource context, pollution prevention context, management contexts, etc.). The task comprised a number of sub-tasks:

- The **NWA** was carefully examined to identify **contexts** that could potentially benefit from information from toxicity tests. Considerable insight was obtained during discussions on how DWAF approaches the implementation of the NWA. For example, much emphasis is placed on the nature of the water use, *e.g.* as categorised in the South African Water Quality Guidelines (1996). 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 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.
- A series of **management criteria** were identified and defined. These included **generic management criteria** and other criteria. 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.

The task 'Compile inventory of tests' focused on the selection of the full range of toxicity tests that could be considered for the guide. Appropriate toxicity test criteria were also selected and defined. The final step of the task required that the appropriate information was captured for each toxicity test for all the identified criteria.

The task 'Integrate information' involved the integration of the specific management criteria of the various management contexts and the available list of toxicity tests to produce a list of toxicity tests appropriate to each management context. The water quality management criteria were seen as the critical link between the management contexts and the inventory of tests. The management contexts and inventory of tests were thus developed as independently as possible (*i.e.* no cognisance was taken of the management contexts when selecting the toxicity tests for the inventory).

For the final task 'Produce guide' it was necessary to capture all the information in a user-friendly format that could be effortlessly accessed and understood by a wide range of users (including water resource and source managers, water users, researchers and educators).

4. Summary of Information Produced and Captured (Results)

Water quality management framework

The following **NWA contexts** 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; and

National status and trends monitoring. Source context – Pollution prevention; Emergency incidents; and Licence conditions.

The **generic water sources** that were identified include the water resource types, inland water and estuarine water. Each of these is divided into zones, namely water body, sediment and groundwater.

The **generic management criteria** that were defined for the management contexts (NWA contexts and generic water sources) are as follows: Legally defensible (classification – yes or no); Effect manifestation period (classification – short-term or long-term); Target kingdom (classification – animal or plant); and Maximum turnaround time (days).

Other management criteria that were identified are: 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) and sample properties (*e.g.* very dark colour, etc.) that can interfere with toxicity tests (interferences) (classification – yes or no).

The most appropriate **criteria classifications** for each generic management criterion (for both the resource and source contexts) were captured in two spreadsheet matrices. An example of criteria classifications for three of the NWA contexts for inland water resources are shown in the table:

NWA context	Reserve – Humans	Monitoring ecosystem health	National status and trends monitoring
	Test for inland water resource water body		
Legally defensible	Yes	Any	Any
Effect period	Any	Any	Long-term
Target kingdom	Animal	Any	Any
Max days turnaround	30	60	60

In effect, this task produced a series of water quality management contexts, each with well-defined management criteria for choosing toxicity tests.

Inventory of toxicity tests

The toxicity tests and test criteria were captured in a spreadsheet matrix, and each cell in the matrix was populated with the required information. More than 100 toxicity tests are listed. A range of test organisms/test material are included, namely fish, frogs, molluscs, echinoderms, coelenterates, crustaceans, insects, water plants, algae, protozoa, bacteria, fungi, animal cells and enzymes. Tests are applicable to the aquatic and estuarine environments and are used for the protection of aquatic life as well as human health. Tests display a range of acute (short-term) (*e.g*. lethality, respiration, luminescence, etc.) and chronic (long-term) (*e.g*. growth, reproduction, behaviour, molecular changes, etc.) responses. The majority of tests selected are single species laboratory tests. However, a few

active monitoring (*in situ)* tests using biomarker measurements (*e.g*. measurement of mixed function oxidase in bivalves) are also included.

Two sets of criteria were included in the spreadsheet matrix for which information was required, namely water quality management criteria and supplementary criteria. The water quality management criteria include the generic criteria and other criteria mentioned under 'Water quality management framework', as well as criteria related to generic types of water sources. Supplementary criteria addressed 'appropriate uses' and technical issues such as test method origin and references; type of exposure; test material/organism; test container; measurement endpoint and parameter; exposure period; toxicity test endpoint; detection limit; access to organism/material; organism maintenance; and infrastructure and equipment.

The information provided for the water quality management criteria satisfied the classifications provided in the 'Water quality management framework', *e.g.* legally defensible – yes or no; interferences, for example very dark colour – yes or no; etc. The 'appropriate uses' criteria included information on whether or not (yes or no) the test can be applied for a specific water use, namely ecosystem integrity, domestic use, irrigation, livestock watering and aquaculture.

In summary, this task produced a master list of toxicity tests with sufficient information associated with each to satisfy the requirements of the integration task.

Integration of information

For all the water quality management criteria, the matching process is carried out automatically. How the matching is done is explicit in the rules (and guiding principles) applied to establish the management criteria in NWA contexts (Water quality management framework), and in the way the information is captured for individual toxicity tests (Inventory of tests) according to these criteria. Those tests that match all the criteria in each management context are deemed to be the most sensible tests to perform. The following general approach to matching is taken.

- *Compulsory requirements*. An exact match between all the compulsory management requirements is enforced. The user has no control over these other than by defining the NWA context, water resource type, and zone. These criteria are defined in the management criteria matrices.
- *Situation-specific requirements matched automatically.* The user may also specify certain other requirements that only serve to shorten the list of toxicity tests based on the management criteria. Specifying these requirements is not mandatory.
- *Situation-specific requirements matched by user.* For some requirements (supplementary information), it is not appropriate to attempt automatic matching. In these cases, the guiding principles are provided for the user's consideration but not enforced in the spreadsheet interface. The user, therefore, decides which of the recommended tests are appropriate.

Guidance facility

The initial idea with the final task 'Produce guide' was to produce the guide in the form of a paper document. However, because of the multi-dimensional nature of the information, and because the management contexts and inventory of tests were compiled in spreadsheets, the most appropriate means to produce the guide was in spreadsheet format. Therefore, as alternative to a paper document an electronic facility (in Excel) was produced.

There are 13 worksheets in the guidance facility. These are designated: 'Background'; 'Instructions'; 'Resource Context'; 'Resource Tox. Tests'; 'Source Context'; 'Source Tox. Tests for Discharge'; 'Source Tox. Tests for Resource'; 'Resource Management Criteria'; 'Source Management Criteria'; 'Resource MM'; 'Source MM Discharge'; 'Source MM Resource'; and 'Inventory of tests'.

The worksheets 'Resource Context' and 'Source Context' list the specific NWA contexts, the water resource type, the zone, water resource water quality, protection required and optional criteria.

The list of recommended tests that are produced when stating the requirements in the 'Resource Context' and 'Source Context' worksheets are captured in the corresponding worksheets 'Resource Tox. Tests', 'Source Tox. Tests for Discharge' and 'Source Tox. Tests for Resource'.

The worksheets 'Resource Management Criteria' and 'Source Management Criteria' contain the criteria classifications for each management criterion (Water quality management framework). The 'Resource MM', 'Source MM discharge' and 'Source MM resource' worksheets (MM = 'Match Matrix') are matrices that store marks (or flags) that indicate whether individual criteria for each toxicity test satisfy the specified user requirements. The last worksheet 'Inventory of toxicity tests' contains all the toxicity tests, criteria and information captured.

5. Conclusions and Recommendations

- The project team succeeded in meeting the objective of the study, namely to establish a guide for the selection of toxicity tests that support the information requirements of the NWA.
- An interactive and well-structured process enabled the identification of an appropriate suite of management criteria that could serve as the critical link between the NWA management contexts and the inventory of tests.
- Inputs from local as well as international scientists assisted in the production of an inventory of tests that contains a wide range of well-established toxicity tests with sufficient information associated with each test to satisfy the information requirements of the NWA management contexts.
- Because of the multi-dimensional nature of the information requirements, the guide was produced as an electronic spreadsheet facility.

- The production of a spreadsheet facility, instead of a paper document, ensured that the guide is available in a compact, user-friendly, format.
- There is no complex interfacing involved in the spreadsheet facility. The guide can, therefore, be effortlessly accessed and understood by a wide range of users, including water resource and source managers, water users, researchers and educators.
- The spreadsheet facility has been developed in a highly modular way and contains a number of worksheets. This does not only improve objectivity, but also makes updating of the facility relatively straightforward.
- The built-in filtering capabilities of the spreadsheet allow the automatic matching of information in different worksheets. Successive filtering can also produce shorter information lists.
- The management criteria of the various management contexts are automatically matched with the available list of toxicity tests to produce a list of toxicity tests appropriate to each management context. This has the advantage that objectivity is imposed on the choice of tests.
- Automatic matching is not possible for all the requirements (*e.g.* supplementary information). This provides the user the opportunity to reduce the list of tests that was automatically generated to suit his/her specific requirements. The specific requirements may include:

    - ❖ toxicity tests that protect specific organism groups;
    - ❖ toxicity tests for direct, pore water and water extract sediment testing;
    - ❖ tests that are reasonably priced;
    - ❖ tests that have a certain turnaround time;
    - ❖ tests only applicable to organic solvent extracts; and
    - ❖ tests only used as definitive tests.

- The toxicity tests recommended by the guidance facility are tests that **can** be carried out, not **must** be carried out.
- The inventory of tests includes local as well as international tests. The recommended list of tests will, therefore, also display a mixture of these groups of tests. Some of the local tests are carried out routinely in local toxicity test laboratories. The user's requirements should be discussed with a biotoxicologist. If required, a recommendation on a reputable international testing laboratory can be made by the biotoxicologist.
- The inventory of tests includes a wide range of tests that has the potential to address a variety of eventualities. It was, however, not possible to include every possible toxicity test. Furthermore, in some instances only a limited number of tests have been developed that use specific test organisms, *e.g.* aquatic plants, or only a limited number of tests are applicable to certain waters, *e.g.* estuarine water. It is, therefore, possible that a situation may arise where the user selects a combination of management contexts and criteria that could result in a very limited list of recommended toxicity tests, or no toxicity tests are recommended. In such a case the user's requirements should be discussed with a biotoxicologist.

- An important function of the guide is to identify gaps with reference to specific tests for specific information requirements. If such gaps are identified, it should be followed up with the Department of Water Affairs and Forestry or the Water Research Commission (WRC), to ensure that tests for that specific purpose are developed.
- The functioning of the guidance facility has been evaluated extensively. It is, however, possible that a specific combination of management contexts and criteria do not provide a satisfactory list of tests, or the expected list of tests. In such a situation the matter should be addressed with a biotoxicologist, or the custodian/developers of the guidance facility can be contacted.
- It is expected that the guidance facility will be a valuable educational tool. It is hoped that it would stimulate interest amongst biological students to follow a career in aquatic toxicology and that it would sensitise water resource and source managers to the importance of toxicity testing in water quality management.
- It is recommended that the WRC acts as custodian of the guidance facility. This entails that any updating or expansion of the facility is arranged and guided by the WRC. The latest version of the facility will, therefore, be available from the WRC.

6. Archiving of Information

All the information collected, produced and integrated during the study is captured in the electronic Guidance Facility. **A copy of the electronic guide is enclosed in CD format in the back of the report.**

ACKNOWLEDGEMENTS

The production of this report and the associated guidance facility was funded by the Water Research Commission. The authors wish to thank the Steering Committee Members and Project Members very sincerely for their contributions.

Steering Committee Members:

Mr HM Du Plessis	Water Research Commission (Chairman)
Dr SA Mitchell	Water Research Commission
Ms T Hopkins	DWAF
Mr A Manamela	DWAF
Ms T Zokufa	RQS, DWAF
Mr J Dabrowski	RQS, DWAF
Ms L Coetzee	Rand Water
Dr CWS Dickens	Umgeni Water
Ms MJF Krüger	Midvaal Water Company
Mr AS Louw	ERWAT, Scientific Services
Ms A Chapman	ERWAT, Scientific Services
Mr M Claassen	Environmentek, CSIR
Dr JA Day	FRU, University of Cape Town
Dr HL Malan	FRU, University of Cape Town
Ms S Bollmohr	FRU, University of Cape Town
Prof OS Fatoki	University of Venda

Project Members:

Dr AL Kühn	RQS, DWAF
Dr S Jooste	RQS, DWAF
Mr PJ Herbst	DWAF
Ms S Oelofse	DWAF
Mr P Viljoen	DWAF
Dr H du Preez	Rand Water
Ms EA Venter	Environmentek, CSIR
Ms S Pillay	Environmentek, CSIR
Ms H Pearson	Ecosun
Dr V Wepener	RAU
Prof G Steyn	RAU
Prof JH van Wyk	University of Stellenbosch
Dr N Muller	University of Rhodes
Dr C Blaise	Environment Canada
Dr RP Scroggins	Environment Canada

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY

Abiotic. Non-living component of an ecosystem.

Active monitoring. Placing test organisms maintained in the laboratory in containers in the field. Also known as *in situ* testing.

Acute. Refers to a stimulus that induces an effect in a short period of time, considered here to be within 96 h. The effect can be lethal or sub-lethal.

Amphipod. Small, generally visible, shrimp/flea-like crustacean (sometimes known as scud) that is part of plankton and feeds on algae and detritus (*e.g.* sea lice, sand fleas, shrimps).

Aquifer. .Defined in the National Water Act (NWA) as a geological formation which has structures or textures that hold water or permit appreciable water movement through them.

Barrel weed. A small (3 to 8 cm long) red gelatinous marine alga, with short alternate tapered and densely matted branches. It often attaches itself to larger seaweeds.

Biomarker. An indicator in a living organism that reflects sub-lethal molecular and/or cellular changes (biochemical, physiological and histological) occurring along a metabolic pathway because of exposure to chemical or physiological changes.

Biotic. Living component of an ecosystem.

Carcinogen. A toxicant that causes cancer.

Catchment. In relation to a watercourse means the area from which any rainfall will drain into the watercourse through surface flow to a common point.

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

Chronic. Refers to a stimulus typically occurring in small doses that induce direct or cumulative sub-lethal effects over a long period of time.

Coelenterate. Radially symmetrical aquatic animal having a saclike body with a central mouth, usually surrounded by tentacles with stinging cells (*e.g*. hydra, jellyfish, anemones).

Crustacean. Fresh- and saltwater animal having no backbone, with a segmented body and jointed legs and a hard chitinous exoskeleton (*e.g*. crabs, shrimps, water fleas).

Definitive test. An experimental technique that estimates the concentration of the toxicant at which a specified percentage or number of organisms exhibit a certain response. Typically reports a toxicity endpoint, *e.g.* lethal concentration (LC), effect concentration (EC), inhibition concentration (IC), no observed effect concentration (NOEC), etc.

Duckweed. A small flowering plant that floats on or near the water surface in a bright green layer. The plant has no stems or leaves. The plant body consists of a green sphere, called a frond, with tiny roots.

Echinoderm. Marine invertebrate covered with calcite plates or spines. It usually has a five-fold radial symmetry and can be free-swimming or be attached to the sea bottom (*e.g*. sea urchins, seastars, sand dollars).

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

Effect concentration (EC). The estimate of the toxicant concentration at which a specified percentage of the test organisms or material would be affected, *e.g.* the EC_{10} or EC_{50}.

ELISA. Enzyme linked immunosorbant assay.

Endocrine disruption. The extent to which a toxicant mimics natural hormones, inhibits the action of natural hormones or alters the normal regulatory function of the immune, nervous or endocrine systems.

Estuary. Defined by the NWA as a partially or fully enclosed body of water which is permanently or periodically open to the sea, and within which the sea water can be diluted, to an extent that is measurable with freshwater drained from land.

Flow-through test. A test in which the water is continuously renewed by the constant inflow of freshwater. Also known as continuous-flow.

Fungus. Eukariotic organism that lacks chlorophyll and feeds on decomposing organic matter (*e.g*. yeasts, molds, mushrooms).

Heavy metal. A metallic element with atomic number greater than 20 (*i.e.* that of calcium)

Inhibition concentration (IC). The estimate of the toxicant concentration at which a specified percentage of the test organisms or material would be inhibited, *e.g.* the IC_{25} or IC_{50}.

IR. Induction ratio.

In situ. In the natural environment.

In vitro. Outside a living organism, in an artificial environment.

Lethal concentration (LC). The estimate of the toxicant concentration at which a specified percentage of test organisms die, *e.g.* the LC_{10} or LC_{50}.

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

Long-term. The period of concern for the manifestation of lethal or sub-lethal toxic effects is weeks or more (a significant portion of the life span of the organism).

Lowest observed effect concentration (LOEC). The lowest tested toxicant concentration that causes a statistically significant sub-lethal effect on test organisms.

Mayfly. Fragile insect with delicate membranous wings that develop in freshwater (larval stage) and live very briefly as a terrestrial adult (less than two days).

MCIG. Minimum concentration to inhibit growth.

Midge. Very small mosquito-like two-winged insect lacking biting mouthparts (*e.g.* chironomids). The larval life stage (larvae often C-shaped and have spastic, squirming movement) is aquatic.

Mollusc. A salt- or freshwater invertebrate having a soft unsegmented body, usually enclosed in a shell (*e.g.* snail, clam, bivalve).

***MR*.** Mutation ratio.

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

Mysid. Small shrimp-like marine crustacean with a carapace over most of the torax. The female carries her eggs in a pouch beneath the thorax.

No observed effect concentration (NOEC). The highest tested toxicant concentration that does not cause any statistically significant sub-lethal effect on test organisms. Alternatively known as the NEC (no effect concentration) or the NOAEC (no observed adverse effect concentration).

NWA information requirements. These define (as accurately as necessary) the data, information or decision support required from toxicity test results in a particular NWA context by the relevant organisation defined or implied by the Act.

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

Pore water. The water occupying the space between sediment particles. Also called interstitial water.

Protozoan. Small and simple single-celled motile eukaryotic (*i.e.* possessing a well-defined nucleus) animal that can perform all the necessary metabolic and reproduction functions (*e.g.* amoebas, ciliates, flagellates).

Range finding test. Toxicity test carried out on ten-fold dilutions of a sample

Reserve. Quantity and quality of water required.

RP. Relative potency.

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

Semi-static test. A toxicity test in which the test water is renewed (replaced) periodically, *e.g.* every 24 h. Also called 'batch replacement', 'static replacement', 'static renewal' or 'renewal'.

Short-term. The period of concern for the manifestation of lethal or sub-lethal toxic effects in days or less (a short period in relation to the life span of the organism).

Standard test: A toxicity test that is widely accepted by the scientific community. Specific requirements include: (a) procedures should have a sound statistical basis and should be repeatable and generate similar results in different labs (standardised and defined protocols are used); (b) data should include effects of a range of concentrations within realistic durations of exposure (should be quantifiable through accepted methods of evaluation); (c) test should have some field predictive capability for similar organisms; (d) data should be useful for risk assessment; (e) test should be sensitive and realistic; and (f) test should be economical and easy to perform.

Static test. A toxicity test in which test water is not renewed during the test.

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

Target kingdom. The kingdom of organisms (either plant or animal) of concern that is to be afforded some level of protection.

Target system. The biological system (humans, fish, plants, etc.) of concern that will potentially manifest one or more toxic effects.

Teratogen. A toxicant that causes the induction of permanent structural malformations or defects in offspring following prenatal administration to the mother. (Thalidomide is a well-known teratogen).

Test organism. The biological (typically a specific species), cellular or sub-cellular system (typically associated with a specific species) that is used in a toxicity test.

TI. Teratogenic index.

Toxic effect. An effect that manifest as an impairment of the activity of the organism or the cellular or sub-cellular system. These effects are also limited to those that can be detected, either currently or potentially, locally or internationally, by a 'toxicity test'.

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

Toxicity test. A toxicity test is regarded an experimental procedure that measures, under defined conditions in the laboratory or in the field, the toxic effects of chemical pollutants in water on a group of living organisms or a cellular or sub-cellular system. The 'water' may be an existing water source, a sample thereof or an extract or leachate.

Vitellogenin (VTG). A classic steroid-induced protein. If an oviparous vertebrate is exposed to estrogen, it will synthesise VTG. VTG is synthesised in the liver under the control of estrogen. This usually occurs in females but VTG synthesis can also be induced in males. VTG is taken up into the oocyte by receptor-mediated endocytosis. It is present in the plasma of females several months before ovulation.

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

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

Water resource. Defined by the NWA as including a watercourse, surface water, estuary or aquifer.

1. INTRODUCTION

1.1 Background and Motivation

The Constitution of the Republic of South Africa (1996) states the following in the Bill of Rights.

> **Bill of Rights**
>
> 24. Everyone has the right –
>
> a. to an environment that is not harmful to their health or well-being; and
>
> b. to have the environment protected for the benefit of present and future generations, through reasonable and legislative and other measures that –
>
> i. prevent pollution and ecological degradation;
>
> ii. promote conservation;
>
> iii. secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development.

This statement is endorsed by the National Water Act (NWA) that was promulgated in 1998. The NWA introduced the concept of a Reserve, which is a novel idea in water resource management, not only nationally, but in some respects internationally. The concept of a Reserve as a quantity and quality of water for the provision of basic human use and for the maintenance of sustainability of the aquatic ecosystem, is new in terms of the status it has under the law. The basic human needs reserve provides for the essential needs of individuals served by the water resource in question and includes water for drinking, for food preparation and for personal hygiene. The ecological reserve relates to the water required to protect the aquatic ecosystems of the water resource.

The Department of Water Affairs and Forestry (DWAF) gives effect to the Reserve by means of two sets of administrative tools, namely resource-directed measures and source-directed controls. The resource-directed measures set objectives for the desired level of protection of the resource, and include a resource classification system that requires the grouping of significant surface water resources into protection classes, each of which represent a similar risk of damaging the resource beyond repair (Jooste, 2000). Each class has objectives for water quality, quantity and assurance, habitat structure and biota. The source-directed controls aim to control what is done to the water resource as a result of activities on land in order to achieve the protection objectives (DWAF, 1997). The NWA states that the person who owns, controls or uses the land in question is responsible for taking measures to prevent pollution of water resources. The source-directed controls include source reduction measures that aim to reduce or eliminate the production of pollutants which could harm the water resource. Source-directed controls will make use of permits and standards while promoting changes in technology and land-use (Jooste, 2000).

A crucial implication of the NWA is an effect-based approach to resource management (Jooste, 2000). This effect-base is apparent in both the resource-directed measures and the

source-directed controls. Within the context of the regulatory use of toxicity assessments in water resource management in South Africa, effect assessments could be applied within a law enforcement context, namely to set standards used in source-directed controls. An effect 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.

Biological toxicity tests are ideally suited to assess the effect of stressors. A large number and variety of toxicity tests are internationally available for aquatic toxicity testing (Slabbert et al., 1998a,b; Wells et al., 1998; Persoone et al., 2000; Blaise et al., 2005). A range of toxicity tests have also been established for South African use. These include several laboratory-based short-term tests (Slabbert et al., 1998a,b; Slabbert and Venter, 1999; Pool, 2000; Slabbert, 2004; Whittcut et al., 2004) as well as artificial stream systems (Scherman and Palmer, 2000). Biomarkers aimed at the detection of biochemical, physiological, behavioural and histological effects of toxic chemicals in laboratory and field applications (Venter et al., 2004) and methods for the detection of estrogen mimicking activity in water (Slabbert et al., 1999; Hurter et al., 2002) have also received extensive attention. Most of the local tests are applied in hazard assessments to establish toxicity at the source level.

Our situation in South Africa 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.

1.2 Objective

The objective of this project was to establish a guide for the selection of toxicity tests that support the information requirements of the National Water Act.

In order to clarify the objective, specific phrases are highlighted:

- ‘guide’ refers to a manual, tool or mechanism.
- ‘for the selection of toxicity tests’ means that this guide is required to assist in choosing appropriate toxicity tests.
- ‘toxicity tests that support the information requirements of the National Water Act’ means that these tests will specifically focus on the information that is required by the National Water Act.
- ‘information requirements of the National Water Act’ refers to phrases, descriptions, objectives in the Act that intimate the need for data, information or decision support from toxicity tests.

1.3 Approach to the Study

A highly consultative and interactive process was followed during the execution of the project. Both scientists and water quality managers (DWAF) participated in the project. Scientists represented local institutions such as universities, Rand Water, and CSIR, as well as consultancies (Ecosun and Insight Modelling). International co-workers (*e.g*. Environment Canada) also made substantial contributions to the project. The role of the scientists was to share their knowledge and practical experience in toxicity testing. Project members from DWAF were required to provide inputs into management aspects and approaches related to the NWA and the South African Water Quality Guidelines (1998).

The project was executed in distinct tasks, namely:

- Plan project;
- Analyse regulatory framework;
- Compile inventory of tests;
- Integrate information; and
- Produce guide.

As part of project planning, a core project team (CSIR and Insight Modelling) met a number of times with participants from DWAF to discuss the boundaries and requirements of the project, and to confirm the structure for project execution. It was agreed that for the regulatory framework, the existence of actual toxicity tests to satisfy the identified requirements was deemed irrelevant. The existence of necessary infrastructure and capacity to perform the tests and to assess and report results were also deemed irrelevant. The focus was on the type of information that toxicity tests can provide in terms of NWA requirements. The advantage of this was that the ultimate guide will identify gaps in current capacity and thus help focus future research and development. Concerns were raised about

- Possible subjectivity by project team members in recommending the most suitable toxicity tests for inclusion in the guide; and
- Possible subjectivity of the ultimate users of the guide.

The first concern was addressed by executing the project in separate tasks. The second concern could be solved by presenting the final guide in a format that would limit subjectivity.

Two subsequent workshops were held with participants from DWAF to obtain inputs into management requirements, water sources considered important and envisaged information requirements. The process proved particularly difficult, probably because of the inability of the core team to bridge the divide (at the time) between the team's understanding of the NWA and its implementation, and DWAF's understanding of toxicity tests and the kind of information they can provide. Core team (CSIR and Insight Modelling) meetings were held at regular intervals until all the tasks related to the water quality management framework was completed. A workshop was also held with the group of scientists involved in the project on the proposed inventory of toxicity tests, to initiate the task and plan the participation and

contributions. Hereafter, the task was carried out *via* electronic communication and one-on-one meetings. The information integration and development of the guidance facility was mainly carried out by the core team. The guidance facility was circulated to project members and interested biotoxicologists (local and international) for comments and improvement.

The research report summarises the process followed and the decisions taken to establish the guidance facility. It is hoped that this not only explains why things were done the way they were, but contributes to the overall transparency of the project.

A short summary of the scope of toxicity testing is given in Chapter 2. The term 'toxicity test' often has different meanings for scientists and water quality managers. To ensure a common understanding, the term was clearly defined. The features that are excluded from toxicity tests are listed and the extent of use is discussed.

At the onset of the task 'Analyse regulatory framework' a few decisions were taken. It was felt that the term 'regulatory' was somewhat misleading since regulations *per se* comprise only one of the many parts that exist within the NWA. The term 'regulatory' was thus changed to 'water quality management'. Furthermore, the NWA consists of various chapters, parts and sections. To refer to parts of the NWA that could benefit from information from toxicity tests was confusing, when information from chapters and sections were also considered. It was, therefore, decided to use the term 'context' instead of 'part' (*e.g.* NWA context, resource context, pollution prevention context, management contexts, etc.). The various steps taken and decisions made to analyse the water quality management framework are discussed in Chapter 3. Firstly, the NWA was carefully perused to identify **NWA contexts** that could benefit from information from toxicity tests. For additional water quality management information, the Water Quality Guidelines were examined. **Generic water sources** that could conceivably be subjected to toxicity tests were also identified. A series of **management contexts** were then identified involving both the identified NWA contexts and the generic water sources. Once the management contexts were identified, appropriate **management criteria** were identified and defined. These included **generic criteria** and other criteria. Finally, each of the broader management contexts, namely the resource and source contexts, was allocated an appropriate classification for each generic management criterion. The management contexts and criteria classification matrices were captured in spreadsheets.

The first part of the task 'Compile inventory of tests' focused on the selection of the full range of toxicity tests that could be considered for the guide. Only well-established tests were selected. Aquatic toxicity tests as well as those used for human health protection were included. Human health-related tests were, however, restricted to those used in the water environment (typical mammal tests were omitted). Appropriate toxicity test criteria were also selected and defined. The final step of the task involved capturing the toxicity tests and the criteria (toxicity test criteria as well as the identified water quality management criteria) in a spreadsheet and populating the matrix with sufficient information associated with each test to satisfy the requirements of the integration task. The steps followed and the decisions made to compile the inventory of tests are discussed in Chapter 4.

The task ‘Integrate information’ involved the integration of the specific management criteria of the various management contexts and the available list of toxicity tests to produce a list of toxicity tests appropriate to each management context. The water quality management criteria were seen as the critical link between the management contexts and the inventory of tests. The management contexts and inventory of tests were thus developed as independently as possible (*i.e.* no cognisance was taken of the management contexts when selecting the toxicity tests for the inventory). The integration of information is discussed in Chapter 5. Those tests that match all the criteria in each management context are deemed to be the most sensible tests to perform. The matching rules are made explicit, as are the guiding principles upon which they are based.

It was extremely important to capture the guide in a user-friendly format that could be effortlessly accessed and understood by a wide range of users (including water resource and source managers, water users, researchers and educators). The initial idea with the final task ‘Produce guide’ was to produce the guide in the form of a paper document. However, because of the multi-dimensional nature of the information, and because the management contexts and inventory of tests were compiled in spreadsheets, the most appropriate means to produce the guide was in spreadsheet format. Therefore, as alternative to a paper document an electronic facility was produced. The structure of the guidance facility is discussed in Chapter 6. Instructions on how to use the facility are also included. **A copy of the electronic guide is enclosed in CD format in the back of the report.**

The conclusions and recommendations of the study are presented in Chapter 7 and the references are listed in Chapter 8.

2. SCOPE OF TOXICITY TESTING

Scientists and managers involved in the area of water quality management have various interpretations of the term 'toxicity test'. In order to ensure that participants in the project and the ultimate users of the guide have a common understanding of the term, it was important to provide an unambiguous definition of a toxicity test. It was also necessary to take cognisance of the features that are excluded from toxicity testing and to highlight the capabilities. Other definitions and principles related to toxicity testing are included in the 'Glossary'.

2.1 Definition of a Toxicity Test

> For the purposes of this project, a **toxicity test**
> 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.

The water referred to in the definition may be an existing water source, a sample thereof or an extract or leachate.

A most fundamental aspect of a toxicity test is that it is primarily focused on measuring the (toxic) **effects** of pollutants, not the existence or concentration of the pollutants themselves. The **chemical pollutants** refer to chemicals that are dissolved, or suspended in solid phases, or adsorbed on biotic or abiotic surfaces, that can produce a toxic effect. These chemicals include metals or metal ions (*e.g.* lead, mercury, iron, manganese, etc.), inorganic chemicals (*e.g.* nitrate, ammonia, sulfate, fluoride, cyanide, etc.) and organic chemicals (*e.g.* phenols, petrochemicals, pesticides, steroids, algal toxins, etc.).

2.2 Features Excluded from a Toxicity Test

The above definition of a toxicity test implies the exclusion of certain capabilities that are sometimes considered to be within the scope of toxicity testing. To avoid confusion, and to ensure that the scope of toxicity testing is properly delineated, capabilities specifically excluded are listed:

- Traditional chemical analytical techniques that measure the presence, or concentrations, of substances (*e.g*. mercury, cyanide, etc.) in the water or in target organisms (*e.g.* fish, molluscs, etc.) are excluded. These traditional chemical techniques include 'wet chemistry methods', or using atomic absorption spectrophotometry, inductively coupled plasma, and a host of others.
- Measurements of bioaccumulation or biodegradation are excluded (because traditional chemical techniques are excluded).
- The **direct** effects of turbidity, pH and temperature are excluded, although these factors can influence the degree of toxicity associated with chemical pollutants.

- The detection of living organisms (*e.g.* faecal coliform bacteria, viruses, parasites, etc.) is excluded.
- Mechanisms of exposure and other issues that actually comprise the domain of risk assessment are also excluded.

It might be noted that such factors as turbidity, pH and temperature can exhibit effects similar to the toxic effects associated with some chemical pollutants. However, tests examining the effects of these factors are usually termed **biological tests**, not **toxicity tests**.

2.3 Application of Toxicity Tests

2.3.1 Test samples

Table 2.1 provides some examples of samples that might exhibit toxic effects and how samples are collected and prepared for toxicity testing. In some cases, the sample may be subjected to an extraction process in the laboratory that removes particular chemical pollutants (like organics) by passing it through a column. This, in effect, allows the pollutants to be concentrated and the subsequent toxicity test made more sensitive.

TABLE 2.1: Examples of samples that can be tested by toxicity tests

Test sample		Sample preparation
Existing water	***Pollution source***	• Water sample taken directly from a water fraction or water body (*e.g.* grab sample of river, impoundment, etc.) and tested for toxicity in a laboratory. • Water sample physically separated from a wet solid fraction (like a sediment), for example, by filtration or centrifugation, and tested for toxicity in a laboratory. • Addition of organism (*e.g.* fish, *Daphnia*) to water *in situ* and testing for toxicity. • Physical removal of an organism (*e.g.* fish, plants, etc.) from an existing water body (*e.g.* river) for subsequent measurement of biological activity in the laboratory.
	✓ Waste discharges (domestic, sewage, aquaculture, agriculture, industrial), ✓ Runoff, ✓ Natural leachate of any solid or liquid waste or stockpile, and ✓ Spillage.	
	Water resource	
	✓ Freshwater watercourse (river, spring, lake or dam) water body, ✓ Freshwater watercourse (river, spring, lake or dam) sediment, ✓ Freshwater wetlands, ✓ Groundwaters, and ✓ Estuaries.	
Solids or liquids	***Pollution source***	• Extract or leachate produced by laboratory **water or solvent** brought into contact with the solid or liquid, then separated from the solid or liquid, and tested for toxicity in a laboratory.
	✓ Solid waste (hazardous, etc.), ✓ Stockpiles (ores, industrial reagents and products), ✓ Chemicals, and ✓ Freshwater watercourse (river, spring, lake or dam) sediment.	

2.3.2 Protection and prediction potential of toxicity tests

Toxicity tests can be used for protection or prediction. Tests are most frequently used in the protection mode. Toxicity tests only employ a limited number of test species compared to

those existing in the environment. When these tests are applied, particularly in battery form, the idea is to provide adequate protection to the wider range of organisms in the environment. The protection mode is particularly applicable in the case of humans, where direct tests are not possible. The selection of tests in such a case would then include organisms and measurements that have proven to be sufficiently sensitive to protect human health or have been specifically developed for this purpose (see chapter 4.3.3.2.1). Sometimes very confident statements are required about potential toxic effects on specific organisms. A typical example is when water quality guidelines are developed. In such cases, confident predictions are necessary. If the test organism is the same as the organism of concern then such predictions can be made. Often this is not attainable because of the wide variety of organism species. It is possible to overcome this limitation by scientifically establishing correlations between the observed effects and effects in the organism of concern.

It appeared evident that only a small subset of toxicity tests appropriate in any particular protective mode will be appropriate in the equivalent predictive mode. It was thus considered appropriate to develop the guide assuming a more lenient protective approach.

3. WATER QUALITY MANAGEMENT FRAMEWORK

The primary aim of the 'Water Quality Management Framework' was to produce information requirements (or criteria) that define the information required from toxicity tests.

The following tasks were carried out (in sequence):

- It was assumed that the information requirements would be primarily determined by the National Water Act (NWA) (1998). The **NWA** was, therefore, carefully examined to identify **contexts** that could potentially benefit from information from toxicity tests. Considerable insight was, however, obtained during discussions on how DWAF approaches the implementation of the NWA. For example, much emphasis is placed on the nature of the water use, *e.g.* as categorised in the South African Water Quality Guidelines (1996). 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 NWA and Water Quality Guidelines various **generic water sources** were identified that could serve as origins of samples for toxicity tests.
- The next task was focused on identifying **management contexts** that would require toxicity test information, involving both the identified NWA contexts and the generic water sources.
- Once the management contexts were identified, it was necessary to identify and define appropriate **management criteria**. These included **generic management criteria** and other criteria. A simple classification was established for each of the generic criteria.
- Finally, each management contexts was allocated an appropriate classification for each generic management criterion.

3.1 Examination of the NWA and Water Quality Guidelines

3.1.1 National Water Act

The NWA consists of 17 chapters that are divided into one or more parts. Each of the parts is further divided into one or more sections. An examination of the index of the Act indicated that four chapters and 15 parts of the chapters appeared to be relevant for information from toxicity tests (Table 3.1). Not all the sections of the parts listed in Table 3.1 were considered relevant. Those sections that could benefit from information from toxicity tests are highlighted in Appendix A.

In order to confirm that information from toxicity tests, and implied by this, that a guide to select appropriate toxicity tests were actually relevant, a detailed examination was carried out on the contents of each part of the listed chapters. It soon became apparent that certain basic requirements were necessary to identify the NWA contexts that could benefit from information from toxicity tests.

- In the first place, the chapters, parts and sections of the Act needed to contain wording or phrases that would obviously benefit from information from toxicity tests, *e.g*. 'impact on a water resource', 'protection of the water resource', 'fitness for use', 'impact on water users', 'impact on water quality', etc.; and
- Secondly, the contents in any part or section of the Act needed to be described or defined in sufficient detail so that the need for information from toxicity tests is clearly demonstrated.

Each part of chapters 3, 4, 5 and 14 are examined in the following paragraphs. A brief description is given for each part. Short discussions are included on whether or not the two requirements mentioned above are satisfied.

TABLE 3.1: Chapters and parts of the National Water Act that are deemed relevant for information from toxicity tests

	Chapter 3: Protection of Water Resources
Part 1:	Classification system for water resources
Part 2:	Classification of water resources and resource quality objectives
Part 3:	The Reserve
Part 4:	Pollution prevention
Part 5:	Emergency incidents
	Chapter 4: Use of Water
Part 1:	General principles
Part 2:	Considerations, conditions and essential requirements of general authorisation and licences
Part 5:	Controlled activities
Part 6:	General authorisations
Part 7:	Individual applications for licences
Part 8:	Compulsory licences for water use in respect of specific resource
Part 9:	Review and renewal of licences, and amendment and substitution of conditions of licences
	Chapter 5: Financial Provisions
Part 1:	Water use charges
	Chapter 14: Monitoring, Assessment and Information
Part 1:	National monitoring systems
Part 2:	National information systems on water resources

3.1.1.1 Chapter 3 of the Act: Protection of water resources

Part 1 requires the development of a classification system for water resources. This classification system may, among other things, establish procedures that are designed to satisfy the water quality requirements of water users as far as it is reasonably possible.

Part 2 requires the actual classification of water resources and the determination of resource quality objectives. The resource quality objectives that are determined may relate, among other things, to –

- the Reserve;
- the characteristics and quality of the water resource and the instream and riparian habitat;
- the characteristics and distribution of aquatic biota; and
- the regulation or prohibition of instream or land-based activities which may affect the quantity of water in or the quality of water of the water resource.

Part 3 requires that the Minister establishes the Reserve for all or part of any significant water resource. The Reserve consists of two parts, namely basic human needs reserve and the ecological reserve. The Reserve refers to both the quantity and quality of the water in the resource, and will vary depending on the class of the resource.

Part 4 deals with pollution prevention and in particular the situation where pollution of a water resource occurs or might occur as a result of activities on land. The person who owns, controls, occupies or uses land is responsible for taking measures to prevent pollution of water resources.

Part 5 deals with emergency incidents such as an accident involving the spilling of a harmful substance that finds or may find its way into a water resource.

Discussion: All the parts of chapter 3 contain one or more phrases that will obviously benefit from toxicity test information, *e.g.* classification system for water resources; water quality objectives for the reserve; basic human needs and ecological reserve; pollution prevention; emergency incidents; etc.

3.1.1.2 Chapter 4 of the Act: Use of water

Part 1 of this chapter deals with general principles. It also defines the so-called ‘section 21 water uses’. Five of the water uses that are defined are listed here.

- Engaging in a controlled activity.
- Discharging waste or water containing waste into a water resource.
- Disposing of waste in a manner that may detrimentally impact on a water resource.
- Disposing in any manner of water which contains waste from, or which has been heated in, any industrial or power generation process.
- Using water for recreational purposes.

Discussion: In respect of part 1 specifically, it is not apparent that toxicity test information is relevant, because the contents are not sufficiently well defined (this part only contains general definitions and principles). However, the listed ‘section 21 water uses’ contain phrases that would obviously benefit from a toxicity test guide.

Part 2 deals with considerations, conditions and essential requirements of general authorisations and licences.

Discussion: The contents do not support toxicity test information because it is not defined in sufficient detail. The interpretation of toxicity test results would certainly be a 'relevant factor' that must be taken into account when considering the issue of general authorisation and licences [for example, to establish 'the likely effect of the water use to be authorised on the water resource and on other water users', namely section 27 (f)]. However, a guide on which toxicity tests need to be used will depend on knowing exactly what waste waters and water resources are involved. Part 2 does not concern itself with the *specifics*, it rather describes the *process.*

Part 3 deals with existing lawful uses; and part 4 focuses on stream flow reduction activities.

Discussion: The contents in parts 3 and 4 do not contain obvious phrases or detailed descriptions that warrant toxicity test information.

Part 5 defines 'controlled activities' and how these are declared.

Discussion: Although engaging in a controlled activity is a 'section 21 water use' (identified to support toxicity test information), the contents in this part in itself do not clearly define the need for toxicity testing.

Part 6 addresses general authorisations that permit the use of water that do not require a licence.

Discussion: This part clearly does not support toxicity test information (no licence required).

Part 7 addresses individual applications for licences; part 8 deals with compulsory licences for water use; and part 9 addresses review and renewal of licences.

Discussion: The trends of the contents in parts 7, 8 and 9 are similar, and they will all three obviously benefit from toxicity test information. Any 'section 21 water use' that potentially affects the resource water quality (obvious phrases) is relevant.

Part 10 deals with failure to comply with authorisations.

Discussion: The contents in this part do not contain obvious phrases or sufficiently detailed descriptions that indicate a need for toxicity test information.

3.1.1.3 Chapter 5 of the Act: Financial provisions

Part 1 addresses the pricing strategy for setting water use charges. There are a number of sections in this part that relate to the quality of waste that may be discharged and the impact on the water quality of the water resource (either of the waste discharge or other use).

Discussion: Contents containing such phrases would potentially benefit from toxicity test information. In effect, the two core phrases that appear in this part are 'pollution prevention' and 'use of water'. These are, however, dealt with in chapters 3 and 4. For simplicity, it is assumed here that little would be gained by trying to distinguish the contents of setting water charges (in this part 1) from the contents identified as relevant in chapters 3 (part 4) and 4 (particularly parts 7, 8 and 9). In other words, in respect of required toxicity test information, those chosen for chapters 3 and 4 will be sufficiently relevant without modification for chapter 5. Therefore, an independent analysis of this part is not necessary.

Part 2 deals with financial assistance, which may be granted by the minister.

Discussion: No contents in this part relate to toxicity test information requirements.

3.1.1.4 Chapter 14 of the Act: Monitoring, assessment and information

Part 1 deals with national monitoring systems. These must provide for the collection of appropriate data and information necessary to assess, among other matters –

- The quality of water resources;
- Compliance with resource quality objectives;
- The health of aquatic ecosystems.

Discussion: The obvious phrases in the text indicate a benefit for toxicity test information.

Part 2 requires the Minister to establish national information systems that may include a water resource quality information system and a groundwater information system. One of a number of objectives of these systems is to provide information to water management institutions, water users and the public on the status of water resources.

Discussion: The contents are sufficiently defined to justify the need for toxicity test information.

Part 3 deals with floodlines, floods and droughts.

Discussion: No description or definition in this part relates to toxicity test information requirements.

3.1.1.5 Summary of the NWA contexts that can benefit from information from toxicity tests

The NWA focuses on two broad water quality management areas that can benefit from toxicity test information, namely the 'resource' (various water resources) and the 'source' (pollution sources). These two broad management areas are also reflected by the Department of Water Affairs and Forestry's (DWAF's) internal operations and infrastructure.

The classification into ‘resource’ and ‘source’, therefore, appeared to be a logic division for the various individual NWA contexts.

Resource related NWA contexts –

- Classification and setting resource quality objectives (chapter 3, parts 1 and 2).
- Reserve determination – basic human needs (chapter 3, part 3).
- Reserve determination – aquatic ecosystems (chapter 3, part 3).
- Monitoring ecosystem health (chapter 14, part 1).
- Monitoring compliance with resource quality objectives (chapter 14, part 1).
- National status and trends monitoring (chapter 14, part 1).

Source related NWA contexts –

- Pollution prevention (chapter 3, part 4).
- Emergency incidents (chapter 3, part 5).
- Licence conditions (chapter 4, parts 7, 8 and 9).

3.1.1.6 *Generic water sources that could conceivably be subjected to toxicity tests*

The NWA relates to two types of water resources that can benefit from toxicity test information, namely estuarine and freshwater. Marine waters are not included in the NWA (their management is currently covered by, amongst others, the National Environmental Management Act (1998)]. Each of the types of water resources can somewhat simplistically be divided into three zones (Figure 3.1). The first zone is the water body. This refers to the bulk water. The second zone is the sediment and refers to the bed of the water resource. The third is groundwater that refers to water present in aquifers.

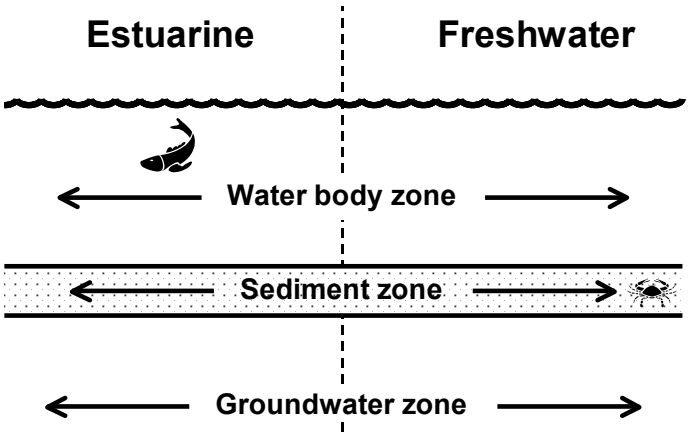

FIGURE 3.1: Illustration of the three zones of estuarine and freshwater

Each zone in each water resource type may require different types of information from toxicity tests. This can arise either because of the way the sample being submitted for toxicity testing is taken (or the way the zone may be tested *in situ*) and because each zone typically has different kinds of organisms dependent on them.

3.1.2 Water Quality Guidelines

DWAF uses 'fitness for use' of the water resource as a primary driver of all water quality management decisions. Seven broad categories of water use are recognised by DWAF. For each of these a set of water quality guidelines have been established (DWAF, 1996). The water quality problems that are typically associated with the various water uses are outlined in the guidelines. The nature of such problems is obviously much broader as those associated with toxicity *per se.* The following paragraphs list the water uses and highlight the toxicity-related problems.

3.1.2.1 Domestic use

'Domestic use' refers to water which is used in the domestic environment. This includes water for:

- drinking;
- food and beverage preparation;
- hot water systems;
- bathing and personal hygiene;
- washing, for example, dishes;
- laundry; and
- gardening, which may include water for fish ponds.

Toxicity-related problems: Health impacts as a result of exposure to chemical pollutants may be short- and long-term.

3.1.2.2 Recreational use

Recreational water refers to all inland water which is used for recreational purposes. Two of the three recreational types defined in the water quality guidelines are relevant in terms of toxicity-related problems, namely:

- Full contact recreation (*e.g.* swimming), and
- Intermediate-contact recreation (involving relatively little water contact).

Toxicity-related problems: Human health impacts can occur through ingestion or skin contact. Exposure can be short- and long-term. Typical human health problems include:

- Skin and ear infections (short-term exposure to chemical pollution);
- Gastroenteric diseases (short-term exposure to algal toxins or other chemicals); and
- Carcinogenic risk (long term exposure to chemical carcinogens).

Very few water quality guidelines are listed for recreational use. Those that are listed are mainly for microbial pollution. Since ingestion can be regarded as 'drinking', the domestic

water chemical pollutants and associated toxic effects can be assumed appropriate for recreational use.

3.1.2.3 Industrial use

In the water quality guidelines, industries are defined as systems of water-using processes. Process types include cooling, steam production, process water, product water, utilities, and wash water. Of all the process waters the utilities water is the only one that can pose toxicity-related problems. This water is linked to domestic use (where water might be used for dinking purposes). This means that human health impacts can occur. This water is classified as used within a category 3 process. This includes 'processes for which domestic water quality is the baseline minimum standard. The water is used in the process without further treatment, or minimum treatment using low to standard technology may be necessary to reach specifications laid down for a desired water quality.'

Toxicity-related problems: The domestic water chemical pollutants and associated toxic effects can be assumed appropriate for utilities water.

It is also conceivable that in, for example, the pharmaceutical industry, products may be produced using water at some point in the process. There may be concerns that this water contains chemical pollutants that may ultimately appear in the product that is produced and hence cause toxic effects in some organisms that use the product. Situations such as these do not preclude toxicity testing. However, the current project restricts itself to those situations in which living organisms are **directly exposed** to the water of concern, namely:

- Organisms (*e.g.* fish, frogs, aquatic invertebrates, etc.) typically occurring in the water itself. These organisms rely on the water itself for survival.
- Organisms (*e.g.* humans, livestock, birds, etc.) typically occurring outside the water itself. These organisms rely on the water for drinking or feeding purposes.

3.1.2.4 Agriculture – irrigation

Irrigation refers to water which is used to supply the water requirements of crops and plants which are not provided for by rain. This includes water for:

- the production of commercial crops;
- irrigation water application and distribution systems;
- home gardening;
- the production of commercial floricultural crops; and
- potted plants.

Toxicity-related problem: Impaired crop quality that may result in inferior products (effect on plants) or pose a health risk to consumers.

3.1.2.5 Agriculture – livestock watering

Water supplies for livestock can originate from impoundments such as dams, from rivers and streams, or from ground water *via* boreholes. The use of water for livestock production depends on several factors, such as the type of production system (intensive or extensive), the type of livestock and the type of livestock products. Two categories of **toxicity-related problems** are listed:

- Livestock consumption – toxicological effects; and
- Livestock product quality – consumer health hazards.

3.1.2.6 Agriculture – aquaculture

Aquaculture is aquatic agriculture. Aquaculture in South Africa can be divided into several sectors;

- cage culture in dams or natural lakes;
- extensive farming in small earthen farm dams;
- extensive and semi-extensive fish farming in purpose designed fish ponds; and
- intensive farming in raceways and tanks.

Toxicity-related problem: Short- and long-term impacts on fish and other organisms as a result of industrial pollution of rivers and the use of herbicides and pesticides.

The water quality guidelines apply only to fish. However, reference is also made to crocodiles and plants (in particular, waterblommetjies), although no guidelines exist at present.

3.1.2.7 Use by components of aquatic ecosystems

Toxicity-related problems: Typical impacts considered in establishing the water quality guidelines included lethality and interference with breeding, feeding, behaviour and migratory patterns.

Consideration was given to providing guidelines in the following ways.

- Target Water Quality Range (TQWR). This is a management objective that is used to specify the desired or ideal concentration range and/or water quality requirements for a particular constituent.
- Chronic Effect Value (CEV). This is used in special cases where the TQWR is exceeded. Setting water quality requirements or objectives at the CEV protects aquatic systems from acute toxicity effects.
- Acute Effect Value (AEV). This is used to identify those cases requiring urgent management attention because the aquatic environment is threatened.

Reference is made to a number of organisms that can be affected in the water quality guidelines, *e.g*. mammals, fish, invertebrates and plants. Guidelines exist for many constituents.

3.1.2.8 Summary of the water uses deemed relevant for information from toxicity tests

Figure 3.2 illustrates the water uses that were discussed in the previous paragraphs. The water uses are grouped in the broad water quality management class 'resource' (paragraph 3.1.1.5). Toxicity-related problems have been identified for all the water uses in the previous paragraphs. In the case of industrial use there is an indirect link with toxicity via the utilities water (can be used for drinking purposes). All the water uses, except the industrial use, are, therefore, considered to benefit from information from toxicity tests. The figure also shows the broad water quality management class 'source' (paragraph 3.1.1.5). Discharges, runoff, spillage and natural leachates are generated during domestic use, industrial use and agricultural use. These, as well as waste tailings from stockpiles (depicted in the figure), are part of the source management class.

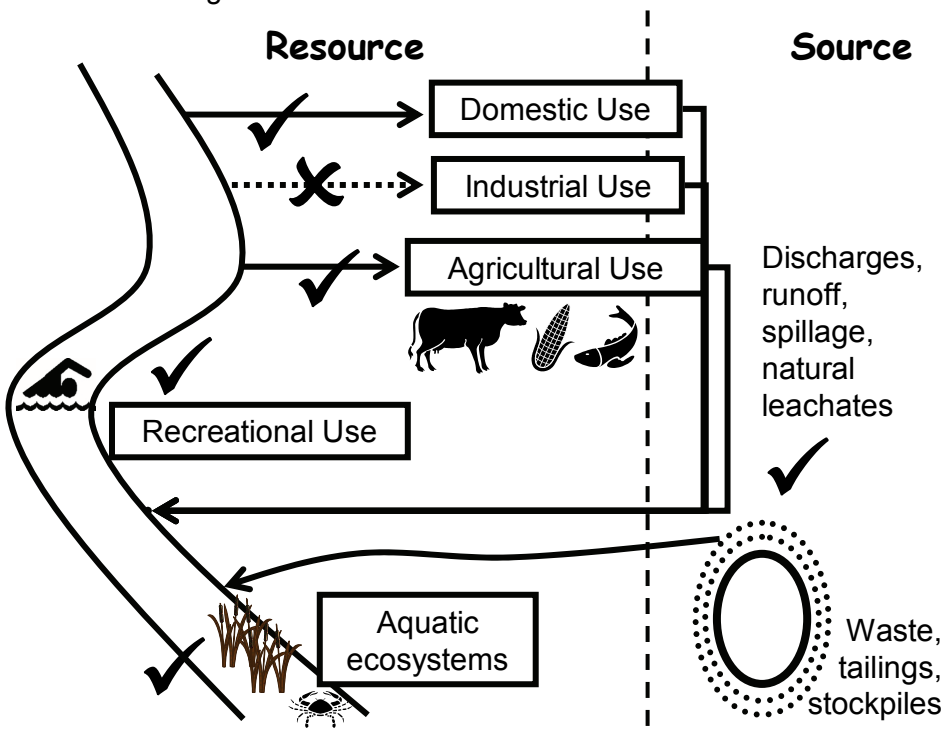

FIGURE 3.2: Illustration of the various water uses that can benefit from toxicity test information (marked with a ✓). Also shown in the figure are various waste discharges and run-off that can be tested for toxicity (marked with a ✓). The figure also shows the resource and source classification

3.1.2.9 Identification of appropriate target groups

'Fitness for use' is often quite naturally associated with a specific group of biological organisms of concern, namely the **target group**. Toxicity tests on the other hand employ specific species from these target groups, called **test organisms**. To facilitate the choice of appropriate target groups (and target kingdoms), simple relationships were established between

- typical target groups and water use (paragraphs 3.1.2.1 to 3.1.2.8), and
- typical target groups and various water resources (paragraph 3.1.1.6).

An examination of the different water uses revealed that each water use can be reasonably well associated with fairly well-defined target groups (Table 3.2). The very nature of a water resource implies a dependence of various organisms on it. Some typical target groups naturally associated with various water resources are listed in Table 3.3. The relationships shown in Tables 3.2 and 3.3 are merely intended to provide very general guidance in situations where explicit site-specific data or information is not available.

TABLE 3.2: Target groups and kingdoms associated with water use

Water use	Most obvious target group	Target kingdom
Domestic	Humans	Animal
Recreational	Humans	Animal
Industrial[1]	Humans	Animal
Agriculture – irrigation	Humans and plants	Animal and plant
Agriculture –.livestock watering	Mammals and birds	Animal
Agriculture – aquaculture	Reptiles, fish, plants	Animal and plant
Aquatic ecosystem organisms	Mammals, birds, reptiles, fish, amphibians, invertebrates, plants	Animal and plant

[1] Regarded as equivalent to domestic use

TABLE 3.3: Target groups and kingdoms associated with various water resources

Water resource[1]	Most obvious target group	Target kingdom
Freshwater watercourse (river, spring, natural channel, wetland, lake or dam) – water body	Humans, mammals, birds, reptiles, fish, amphibians, invertebrates, plants	Animal and plant
Freshwater watercourse (river, spring, channel, wetland, lake or dam) – sediment	Reptiles, fish, amphibians, invertebrates, plants	Animal and plant
Freshwater – groundwater	Humans, invertebrates	Animal and plant
Estuary	Humans, mammals, birds, fish, invertebrates (molluscs, crustaceans, seaweeds), plants	Animal and plant

[1] Definitions for specific terms can be found in the 'Glossary'.

3.1.2.10 Protection versus *prediction*

Having examined the Water Quality Guidelines it was evident that toxicity-related information is relevant in two distinct overarching modes, namely **protective** and **predictive.**

The **protective** mode applies when some target group of organisms needs to be afforded some degree of protection against adverse water quality. This protective mode is implied in the general concept of fitness for use. Ensuring fitness for use means, among other things, ensuring that the water is not harmful. However, toxicity tests using relevant species from a particular target group are often not possible (especially when the target group is humans). Accordingly, the precautionary principle often needs to be applied. For example, this might mean that it is not unreasonable for some concern to be expressed about the potential toxicity of a particular water, *e.g*. drinking water, to humans, when this water has exhibited

toxicity to the fish of a toxicity test. Obviously, if a correlation between the toxicity to the fish and toxicity to humans has been scientifically demonstrated, then the expressed concern carries more weight.

The word **predictive** is being used here to suggest the latter more demanding mode. Sometimes very confident statements may need to be made about potential toxic effects in a chosen target group. A typical example when such confident predictions are required, is when water quality guidelines are developed. If the test organism used in a toxicity test is in the target group, then such predictions can be made. However, if it is not the case, correlations between observed effects in the test organism and effects in the target group need to have been scientifically and independently established.

3.2 Management Contexts

It was soon realised that the information captured in the previous parts of Chapter 3 could be structured in various ways to derive management contexts. A simple structure could be visualised as a single-dimensional matrix with various columns and rows. For example, when visualising the NWA contexts (or target groups) as columns in the matrix and the various sources of water samples (or groupings thereof), or specific chemical pollutants (or classes thereof, for example heavy metals) present in a water sample, as rows of the matrix, each cell of the matrix is then a management context. A simple single-dimensional matrix is illustrated in Figure 3.3. It was also realised that there were many inter-relationships between the information presented in the previous parts of Chapter 3. The most appropriate manner to capture all the different relationships was, therefore, to conceptualise a multi-dimensional matrix. The complexity of such a matrix is illustrated Figure 3.4.

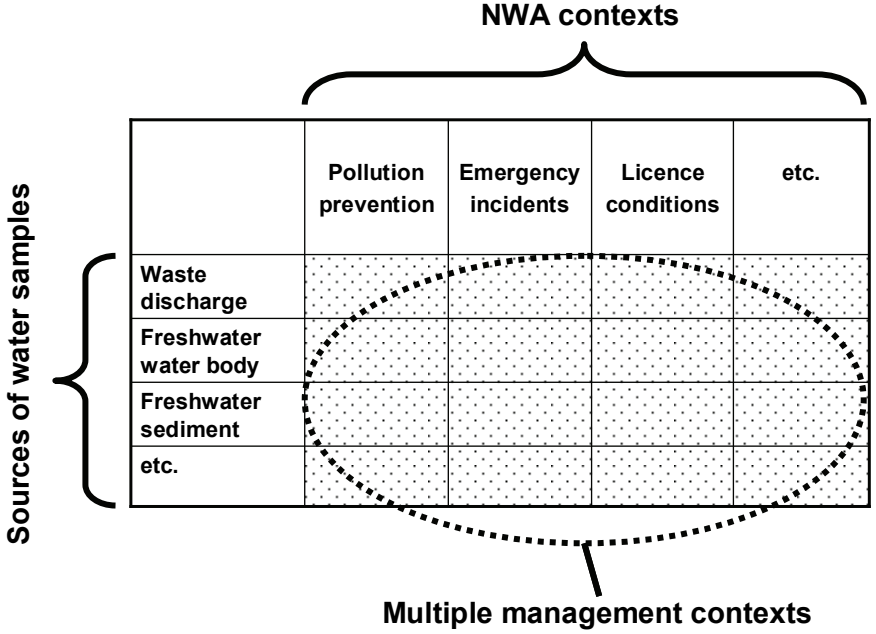

FIGURE 3.3: Illustration of relation between NWA contexts, sources of water samples and management contexts in a single-dimensional matrix

NWA context	Source of water sample	Typical water user	Target group	Management criteria				
				Legally defen-sible	Effect manifes-tation period	Target kingdom	Maxi-mum turn-around time	etc.
Classif. & RQOs	Water body	Domestic, recr, industr	Humans					
Classif. & RQOs	Water body	Aquatic ecosystem	Fish					
Classif. & RQOs	Water body	Aquatic ecosystem	Inverte-brates					
Classif. & RQOs	Water body	Aquatic ecosystem	Birds					
Classif. & RQOs	Water body	Aquatic ecosystem	Mammals					
Classif. & RQOs	Water body	Aquatic ecosystem	Plants					
Classif. & RQOs	Water body	Aquatic ecosystem	Amphi-bians					
Classif. & RQOs	Water body	Agric - irrigation	Humans					

FIGURE 3.4: Illustration of a multi-dimensional matrix converted to a single-dimensional matrix

Since the information requirements are primarily determined by the NWA, the resource and source classes and the various NWA contexts formed the basis of the water quality management matrix and thus largely represented the management contexts.

3.3 Management Criteria

Many of the management contexts, by their very nature, suggest appropriate management criteria. Figure 3.5 illustrates the multiple management criteria for the different management contexts.

Some of the management criteria are such that once the general management context (NWA context and the source of the water sample – see Figure 3.5) is defined, the user has no control over the requirements that must be satisfied. These criteria are called 'generic management criteria' and reflect the perspective of the water resource manager (in particular, a water quality manager) focusing on managing resources (resource context) or someone focusing on managing pollution sources (source context). These criteria are purely managerial and have little to do with other toxicity-related criteria that may be necessary when choosing the actual toxicity tests to perform (chapter 4). The management contexts all have such criteria in respect of choice of toxicity tests for each generic type of water source (*e.g.* freshwater, estuarine, water body, etc. – see paragraph 3.1.1.6). When associated with specific management contexts these criteria place largely non-negotiable demands on the kinds of toxicity tests that can be applied. An important aspect of these criteria is that they represent a very precise link between managerial requirements on the one hand and typical toxicity test considerations on the other. Accordingly, it is crucial to define the criteria in such a way it is relevant and understandable from both points of view.

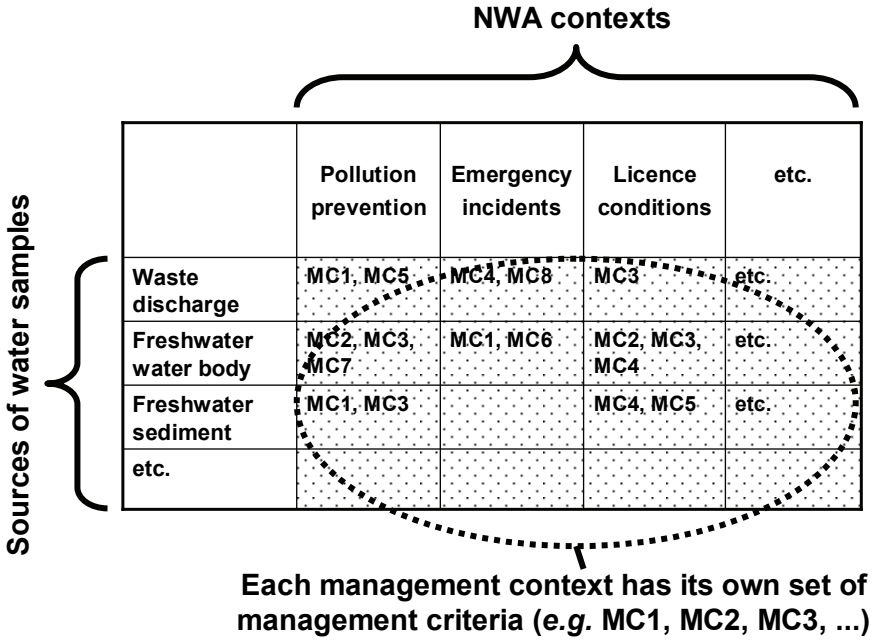

Figure 3.5: Illustration of the multiple management criteria for the different management contexts

As opposed to the generic management criteria where the user has no control over the requirements that must be satisfied, a number of management criteria were identified that provide the user the opportunity to select the requirements that should be satisfied once the general management context has been defined. These criteria are referred to as other management criteria and include the kingdom of organisms a user wishes to afford protection, and optional criteria relating to test specificity and interferences. Choosing these criteria will avoid inappropriate tests to be selected.

3.3.1 Generic management criteria

The following generic management criteria were identified: 'screening *versus* definitive' testing, 'legally defensible', 'effect manifestation period', 'target kingdom', 'maximum turnaround time' and 'acceptable maximum cost'. After some consideration, the last criterion was omitted from the list of criteria. The costs that were assumed were the typical total costs per toxicity test per water sample conducted at an equipped laboratory. These included operating costs (covering consumables, equipment depreciation, etc.) and labour costs. Costs involved all aspects from sample collection through testing to assessment and reporting. The reason for omission was that it was too difficult to capture cost – even if a cost factor or relative costs were used, the listed costs would soon become outdated.

Each management criterion is described in the following paragraphs. Associated with each criterion is a list of classification options that have been used to define the criterion. The implications of the classification for the ultimate choice of toxicity tests are also given. This was necessary to avoid ambiguity during the integration step (chapter 5) where the actual toxicity tests that will best satisfy the requirements will be identified.

Some of the management contexts are such that it is not possible to unambiguously define management criteria. This occurs when the context is such that any classification may apply, depending on site-specific and other constraints at the time. In such cases, the classification 'Any' is used.

3.3.1.1 Screening versus *definitive*

Some contexts typically demand screening tests (that answer the question 'is it toxic?') while others demand definitive tests (that answer the question 'how toxic is it?'). The classification options associated with the criterion 'screening *versus* definitive' and implications for the ultimate choice of toxicity tests are shown in Table 3.4.

TABLE 3.4: Classification options associated with the criterion 'screening *versus* definitive' and implications for the ultimate choice of toxicity tests

Classification	Meaning	Implications
Yes	A screening toxicity test	A screening test must be recommended.
No	A definitive toxicity test	A definitive test must be recommended.
Any	Cannot be classified.	Both screening and definitive tests are permitted.

3.3.1.2 Legally defensible

This is the degree to which the scientific soundness of the experimental procedure can be defended legally. In essence, this means that the experimental result must be reliable. Most importantly, it must be accurate and, to a lesser extent, precise. In other words, the test must actually measure what it claims to measure. The classification options associated with the criterion 'legally defensible' and implications for the ultimate choice of toxicity tests are shown in Table 3.5.

TABLE 3.5: Classification options associated with the criterion 'legally defensible' and implications for the ultimate choice of toxicity tests

Classification	Meaning	Implications
Yes	The experimental procedure of the toxicity test is defensible in court.	The toxicity test experimental results must be reliable. The experimental technique should be recognised as a 'standard method' (*i.e.* be well-defined, well tested and widely used and accepted) and preferably have been used successfully in a court case.
No	The experimental procedure of the toxicity test is not necessarily defensible in court.	The toxicity tests need not be defensible in court but should nevertheless be well established.
Any	Cannot be classified.	Both tests that are defensible and not necessarily defensible in court are permitted.

3.3.1.3 Effect manifestation period

Each management context has a typical 'management timeframe' to which decisions are relevant. It is defined as the period of concern over which toxic effects might manifest themselves. The classification options associated with the criterion 'effect manifestation period' and implications for the ultimate choice of toxicity tests are shown in Table 3.6.

TABLE 3.6: Classification options associated with the criterion 'effect manifestation period' and implications for the ultimate choice of toxicity tests

Classification	Meaning	Implications
Short-term	The period of concern for manifestation of lethal or sub-lethal toxic effects in days or less.	Toxicity tests must be chosen that are appropriate for measuring effects that can manifest themselves in days or less.
Long-term	The period of concern for manifestation of lethal or sub-lethal toxic effects is weeks or more.	Toxicity tests must be chosen that are appropriate for measuring effects that can manifest themselves over weeks or more.
Any	Cannot be classified.	Toxicity tests should be chosen that apply to both short- and long-term effects.

3.3.1.4 Target kingdom

The target kingdom is regarded as the group of organisms to be afforded some degree of 'protection'. The classification options associated with the criterion 'target kingdom' and implications for the ultimate choice of toxicity tests are shown in Table 3.7.

TABLE 3.7: Classification options associated with the criterion 'target kingdom' and implications for the ultimate choice of toxicity tests

Classification	Meaning	Implications
Plant	Toxicity-related information is required specifically for protection of plants.	Toxicity tests should be chosen that indicate potential impacts on plants.
Animal	Toxicity-related information is required specifically for protection of animals.	Toxicity tests should be chosen that indicate potential impacts on animals.
Any	Cannot be classified.	Toxicity tests should be chosen that indicate potential impacts on either plants or animals.

3.3.1.5 Maximum turnaround time

This is the time from receiving at the laboratory a water or solid sample; or a sample, observation or measurement taken at an *in situ* testing site; to assessing and reporting the results of the required test. This includes:

- Sample preparation*, e.g*. filtration, dilution, etc. (inherent to a test) in the laboratory to create the water sample to be tested;
- Organism preparation, *e.g*. overnight culturing; and
- Performing the necessary tests.

The preparation time of some sample extracts and leachates (*e.g.* solid samples, like dry sludge) is not included in the turnaround time of tests, because this is not an inherent part of tests.

The choice of appropriate turnaround time will simply depend on how quickly results are required for the decision support relevant to the management context. For example, in the event of a chemical spill, results will be required quickly. In the case of national status and trends monitoring, a longer timeframe may be acceptable. The classification options associated with the criterion 'maximum turnaround time' and implications for the choice of toxicity tests are shown in Table 3.8.

TABLE 3.8: Classification options associated with the criterion 'maximum turnaround time' and implications for the choice of toxicity tests

Classification	Meaning	Implications
Days	A report must be available within the specified time period.	Toxicity tests are required that must be capable of reporting results within the specified period.

3.3.2 Other management criteria

Once the general management context (NWA context and the source of the water sample) has been specified, the user of the guide can put additional choices into effect to further refine (*i.e.* shorten) the recommended list of tests. This choice of requirements can be made by the user, or can be left to the biotoxicologist.

Definitions for some of the terms used here can be obtained in the 'Glossary'

3.3.2.1 Protection

Target kingdom is one of the generic management criteria (paragraph 3.2.3.1.4) that is determined by the management context. In all the management contexts, except the context 'reserve determination' for basic human needs, both the plant and animal kingdoms are permitted (designated by 'Any') (Tables 3.11 and 3.12). The target kingdom for the context 'reserve determination' for basic human needs is 'Animal'. By including the criterion 'Protection', the user can choose the kingdom of organisms that needs protection. The choice of kingdoms include: 'Any', 'Animal' or 'Plant'. By choosing a kingdom, the classification of the management criteria matrix (Tables 3.11 and 3.12) is overruled. The only classification that cannot be overruled is the kingdom 'Animal' for the context 'reserve determination' for basic human needs. Your choice of kingdom can be based on the intended water use (Table 3.2) or the nature of the water source (Table 3.3).

3.3.2.2 Optional criteria

These criteria refer to the presence of certain toxic chemicals in samples and interferences that can be caused in some toxicity tests as a result of certain sample properties.

3.3.2.2.1 Specificity

The toxic chemical groups for which certain toxicity tests are selectively sensitive include: heavy metals, pesticides, other organics (*e.g.* PAHs), carcinogens, teratogens and endocrine disruptors.

The question that is asked in terms of specificity is: Are you sure no heavy metals, or no pesticides, etc. are present? The choice that the user can make is 'Y=you are sure' or 'N=not sure'. If the user chooses 'Y', the tests that are selectively sensitive to that specific toxic chemical group will be omitted from the recommended list, thus shortening the list. If 'N' is selected, or no choice is made, the tests that are selectively sensitive to that specific toxic chemical group will be included in the recommended list of tests.

3.3.2.2.2 Interferences

The following sample properties can interfere with some toxicity tests: very dark colour, high suspended solids, high salt content, oiliness and soapiness.

The questions that are asked in terms of interferences are:

- Is the sample very dark in colour?
- Does the sample have very high levels of suspended solids?
- Does the sample have a very high salt content?
- Is the sample oily? and
- Is the sample soapy?

The choice that the user can make is 'Y' or 'N'. If the user chooses 'Y' the tests that will show interference to the particular property will be omitted from the recommended list. If 'N' is selected, or no choice is made, no tests will be omitted from the recommended list.

3.3.3 Summary of the water quality management criteria

3.3.3.1 Generic management criteria

The various NWA contexts that can benefit from information from toxicity tests are such that they place very general demands on the type of toxicity tests. The generic management criteria applicable to water quality are summarised in Table 3.9.

TABLE 3.9: Summary of the generic management criteria applicable to water quality management

Management criterion	Classification
Screening *versus* definitive	Screening, Definitive
Legally defensible	Yes, No
Effect manifestation period	Short-term, Long-term
Target kingdom	Animal, Plant
Maximum turnaround time	Days

3.3.3.2 Other management criteria

The other management criteria that allow the user to exercise additional choices to refine the list of recommended tests are summarised in Table 3.10.

TABLE 3.10: Summary of the other management criteria applicable to water quality management

Management criterion		Classification
Protection		Animal, Plant,
Optional	Specificity (heavy metals, pesticides, etc.)	Y
	Interferences (very dark colour, etc.)	Y

3.4 Criteria Classifications for Each Generic Management Criterion

The most appropriate criteria classifications for each generic management criterion, in both the resource and source contexts, are recorded in two separate matrices. These matrices are part of the final guidance facility and are shown in Tables 3.11 and 3.12. The classifications were assigned in close collaboration with project members from DWAF.

In assigning the criteria classifications, basic principles were applied to ensure objectivity and consistency. These are described in the following paragraphs.

A generic principle applied under all circumstances was that each toxicity test included in the inventory of tests must be well established and is assumed to be scientifically sound (see the inventory of tests, chapter 4, for more detail).

3.4.1 Water resource type

In respect to toxicity tests recommended for discharges and, importantly, the generic management criteria identified above, the nature of the resource (freshwater or estuary) into which the discharge might flow (Table 3.12) does not affect the choice of tests (*i.e.* the criteria classifications are the same). Note that this may not be the case for other management criteria. Nevertheless, separate matrices for freshwater and estuaries are retained in the guidance facility in case this decision changes in future.

3.4.2 Screening versus *definitive*

Two types of tests are recognised, screening and definitive. Guiding principles in relation to these are as follows:

- Screening tests are appropriate when the sample is used undiluted and when it is adequate simply to know whether the water is toxic or not.
- Definitive tests are appropriate when a series of dilutions of the sample would typically be tested so that the degree of toxicity can be determined.

The only rule that can be made compulsory and hence automatically implemented in the guidance facility is ensuring a definitive test is recommended for all discharges (only in the source management context). The implementation of the other rules requires the user to specify whether the water is to be used directly or be extracted. The above rules are then applied.

3.4.3 Legally defensible

Legal defensibility is a guiding principle in some management contexts but not in others. The default requirement is that any test is permitted unless the context demands legal defensibility. The latter occurs when the management context, either,

TABLE 3.11: Water resource management criteria matrix

(Normal text are entered manually; criteria in italics are calculated by formulae)

	Inland Water Resources					
National Water Act:	**Chap 3 Parts 1&2**	**Chap 3 Part 3**	**Chap 3 Part 3**	**Chap 14 Part 1**	**Chap 14 Part 1**	**Chap 14 Part 1**
	Class. & RQOs	**Reserve – Humans**	**Reserve – Ecological**	**Monitoring Ecosystem Health**	**RQOs Compliance**	**National Status & Trends**
	Test for Inland Water Resource Water Body					
Legally defensible	Any	Yes	Yes	Any	Yes	Any
Effect period	Any	Any	Any	Any	Any	Long-term
Target kingdom	Any	Animal	Any	Any	Any	Any
Max days turnaround	30	30	30	60	30	60
	Test for Inland Water Resource Sediment					
Legally defensible	*Any*	*Yes*	*Yes*	*Any*	*Yes*	*Any*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Long-term*
Target kingdom	*Any*	*Animal*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*30*	*30*	*60*	*30*	*60*
	Test for Inland Water Resource Groundwater					
Legally defensible	*Any*	*Yes*	*Yes*	*Any*	*Yes*	*Any*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Long-term*
Target kingdom	*Any*	*Animal*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*30*	*30*	*60*	*30*	*60*

	Estuarine Water Resources					
National Water Act:	**Chap 3 Parts 1&2**	**Chap 3 Part 3**	**Chap 3 Part 3**	**Chap 14 Part 1**	**Chap 14 Part 1**	**Chap 14 Part 1**
	Class. & RQOs	**Reserve – Humans**	**Reserve – Ecological**	**Monitoring Ecosystem Health**	**RQOs Compliance**	**National Status & Trends**
	Test for Estuarine Water Body					
Legally defensible	*Any*	*Yes*	*Yes*	*Any*	*Yes*	*Any*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Long-term*
Target kingdom	*Any*	*Animal*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*30*	*30*	*60*	*30*	*60*
	Test for Estuarine Sediment					
Legally defensible	*Any*	*Yes*	*Yes*	*Any*	*Yes*	*Any*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Long-term*
Target kingdom	*Any*	*Animal*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*30*	*30*	*60*	*30*	*60*
	Test for Estuarine Groundwater					
Legally defensible	*Any*	*Yes*	*Yes*	*Any*	*Yes*	*Any*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Long-term*
Target kingdom	*Any*	*Animal*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*30*	*30*	*60*	*30*	*60*

TABLE 3.12: Source management criteria matrix – Discharge into water resource

(Criteria in normal text are entered manually; criteria in italics are calculated by formulae)

	Discharge into Inland Water Resources					
National Water Act:	**Chap 3 Part 4**	**Chap 3 Part 5**	**Chap 4 Part 7, 8 & 9**	**Chap 3 Part 4**	**Chap 3 Part 5**	**Chap 4 Part 7, 8 & 9**
	Pollution Prevention	**Emergency Incidents**	**Licence Conditions**	**Pollution Prevention**	**Emergency Incidents**	**Licence Conditions**
	Test for Discharge			**Test for Inland Water Resource Water Body**		
Legally defensible	Yes	Any	Yes	Yes	Any	Yes
Effect period	Any	Any	Any	Any	Any	Any
Target kingdom	Any	Any	Any	Any	Any	Any
Max days turnaround	30	1	30	30	1	30
	Test for Discharge			**Test for Inland Water Resource Sediment**		
Legally defensible	*Yes*	*Any*	*Yes*	*Yes*	*Any*	*Yes*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Target kingdom	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*1*	*30*	*30*	*1*	*30*
	Test for Discharge			**Test for Inland Water Resource Groundwater**		
Legally defensible	*Yes*	*Any*	*Yes*	*Yes*	*Any*	*Yes*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Target kingdom	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*1*	*30*	*30*	*1*	*30*

	Discharge into Estuarine Water Resources					
National Water Act:	**Chap 3 Part 4**	**Chap 3 Part 5**	**Chap 4 Part 7, 8 & 9**	**Chap 3 Part 4**	**Chap 3 Part 5**	**Chap 4 Part 7, 8 & 9**
	Pollution Prevention	**Emergency Incidents**	**Licence Conditions**	**Pollution Prevention**	**Emergency Incidents**	**Licence Conditions**
	Test for Discharge			**Test for Estuarine Water Body**		
Legally defensible	*Yes*	*Any*	*Yes*	*Yes*	*Any*	*Yes*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Target kingdom	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*1*	*30*	*30*	*1*	*30*
	Test for Discharge			**Test for Estuarine Sediment**		
Legally defensible	*Yes*	*Any*	*Yes*	*Yes*	*Any*	*Yes*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Target kingdom	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*1*	*30*	*30*	*1*	*30*
	Test for Discharge			**Test for Estuarine Groundwater**		
Legally defensible	*Yes*	*Any*	*Yes*	*Yes*	*Any*	*Yes*
Effect period	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Target kingdom	*Any*	*Any*	*Any*	*Any*	*Any*	*Any*
Max days turnaround	*30*	*1*	*30*	*30*	*1*	*30*

- involves, or may involve, legal proceedings (*e.g.* is associated with a water use licence), or
- requires a high level of confidence in the accuracy of test result.

The required legal defensibility in any given management context was assumed to be identical for (Tables 3.11 and 3.12)

- the water body, sediment and groundwater, and
- a given discharge and the associated water resource.

3.4.4 Effect manifestation period

There are two types of effects recognised, short- and long-term. Guiding principles are as follows:

- Short-term tests are appropriate when the effect manifestation period is short.
- Long-term tests are appropriate when the effect manifestation period is long.

There is only one context in which the effect manifestation period is appropriately specified as long-term. This is the 'national status and trends monitoring' context, in the resource management context (Table 3.11). In all other contexts both short- and long-term effects ('Any') are permitted.

3.4.5 Target kingdom

There is only one context in which the target kingdom is appropriately specified as either animal or plant. This context is 'reserve determination' for basic human needs for which the target kingdom is obviously animal (Table 3.11). In all other contexts both the plant and animal kingdoms are permitted.

3.4.6 Maximum turnaround time

The turnaround time is chosen on the basis of either

- the degree of urgency likely to be associated with obtaining results of tests; or
- the degree to which the context requires an 'early warning system'.

The following guiding principles were applied:

Resource management context (Table 3.11) –

- The required turnaround time was identical for a water body, sediment and groundwater.
- Tests performed in a context relating to aquatic ecosystems (like monitoring ecosystem health and national status and trends monitoring) are permitted a longer

turnaround time that those not so related because testing in these contexts is not usually being done as an early warning system.

- Tests performed for ‘classification’, ‘setting resource quality objectives’ and ‘reserve determination’ should be subject to some time constraints because these are important processes that require reasonably speedy completion.

Source management context (Table 3.12) –

- The only context associated with an obvious (and potentially extreme) sense of urgency is ‘emergency incidents’. In this case, although hours might be typically what would be required as a turnaround time, there may be certain scenarios in which waiting for one day for a result may not be unreasonable. For example, local conditions might be such that, although a water resource may soon be threatened, that threat is not immediate.
- Furthermore, in the ‘emergency incident’ context, it can also be envisaged for sediments and groundwater that sometimes threats to the water resource are less serious so that as much as 10 days might occasionally be satisfactory.

It might be noted that relaxing the required turnaround time in some contexts simply allows tests with a slower turnaround time to be included. Tests capable of a turnaround time within hours will obviously appear in the recommended battery of tests. However, the user has a somewhat wider choice, depending on the exact local situation and its specific urgency.

4. INVENTORY OF TOXICITY TESTS

This part of the project involved the production of a master list of toxicity tests, with sufficient information associated with each test to support the water quality management criteria identified for the various water quality management contexts in Chapter 3. In order to avoid possible subjectivity by project team members by favouring certain tests, the inventory was developed without taking cognisance of the specific management contexts that were identified. The following tasks were carried out:

- Selecting toxicity tests for the inventory of tests.
- Selecting and defining the toxicity test criteria to be included in the inventory of tests.
- Compiling the inventory of tests.

A consultative process was followed, obtaining inputs into the different tasks from all the team members. International participation was limited to inputs from Canada, as most of the international experts that were consulted referred the authors to published standard methods. For the first two tasks, a working document with suggestions on toxicity tests to be included in the inventory of tests and proposed toxicity test criteria with definitions and descriptions was circulated to project team members. The inputs and comments were used to create a matrix in a spreadsheet, placing the toxicity tests on the vertical axis and the criteria on the horizontal axis. Project team members were then asked to populate the spreadsheet with toxicity tests and the appropriate toxicity test information.

4.1 Selecting Toxicity Tests for the Inventory of Tests

A guiding principle for the inclusion of tests in the inventory was that they must be 'well established'. To establish the degree to which a test is well established, the following conditions were satisfied.

- The method has gone through extensive testing exercises (*e.g*. to evaluate sensitivity, reproducibility, applicability) and a rigorous write-up (standardised test).
- The method has been published in peer reviewed scientific literature (in sufficient detail to allow the methods to be easily followed in other laboratories).
- The method or similar procedures is used internationally by several laboratories.
- A reasonable cross-section of the relevant scientific community agrees that the method is well established.

Toxicity tests under development were not considered for inclusion in the inventory.

It was agreed to capture as wide as possible a range of toxicity tests in the inventory, namely tests that employ sub-cellular (*e.g*. enzymes) and cellular systems, as well as organisms from various trophic levels (ranging from bacteria to fish). Tests for application in the freshwater as well as the estuarine environment were considered. It was also decided that, where available, to include a range of test species (*e.g*. various fish species). The adverse effects of importance included acute (short-term) (*e.g*. lethality, respiration, luminescence,

etc.) and chronic (long-term) toxicity (*e.g*. growth, reproduction, behaviour, molecular changes, etc.). The majority of tests selected were single species laboratory tests. However, a few active monitoring (*in situ)* tests using biomarker measurements (*e.g*. measurement of mixed function oxidase in bivalves), that have been frequently used and extensively tested, were also considered important for inclusion. Micro- and macrocosm approaches and continuous monitoring systems (*e.g.* fish opercular rhythm reactions) were omitted from the list of tests because the methodologies did not satisfy the conditions stipulated for well established tests.

The focus was on methodologies that are known to local scientists, and that are readily available *via* local information centres and international contacts. Since South Africa is part of the International Organisation for Standardisation (ISO), and many of the well established methodologies from Europe are eventually captured as ISO tests, these well documented tests were seen as an important part of the inventory. The list of tests was extended to standard toxicity test methods used by countries such as the USA, Canada, France and Germany. Most of the French (NF) and German (Deutches Institut für Normung – DIN) methodologies were found to be the same as the ISO test methodologies. It was, therefore, decided to exclude the French tests from the list of tests, and to select only those DIN tests that use different test species. A much wider list of standard toxicity tests than those described by ISO were found to be outlined in USA (EPA) and Canadian (EPS) documents. A number of these methodologies were already in use in local laboratories and it was, therefore, decided to select most of these tests for the inventory. The USA and Canadian tests present most of the methodologies outlined in the well-known American Society for Testing Materials (ASTM) guides. Only the ASTM frog test, not captured in the USA and Canadian methodologies, was thus considered for the inventory. European countries rely heavily on the recommendations of the Organisation for Economic Co-operation and Development (OECD). Therefore, where reference was made to OECD guidelines in methodologies selected for the inventory, the reference for the OECD guideline was also included in the reference list in the inventory. A range of South African tests that have been extensively used and are well documented in WRC and other local reports/theses were also selected for the inventory. Many of the above mentioned test methodologies and results have been published in peer reviewed journals, but for conciseness it was decided not to refer to all the individual publications. (Most of these journal publications can be obtained *via* the internet). A number of standard toxicity tests that have been established in test kit form were also selected for inclusion in the inventory. These test kits are considered important because they are in use in various countries and are currently also accessible by local laboratories. In addition, several important methodologies published in peer reviewed journals that have been applied in South African studies, or have the potential to be used in local laboratories, were also selected for the inventory of tests.

Table 4.1 provides a summary of the toxicity tests and methodologies selected for the inventory of toxicity tests. Detailed information can be found in the final guidance facility. The complete reference list for the methodologies is attached as Appendix B.

TABLE 4.1: Summary of toxicity tests and methodologies selected for inclusion in the inventory of tests

Test Organism/material	Species	Measurements	Methodologies
Fish	Tilapia (*Oreochromis mossambicus*, *Tilapia sparmanii*), guppy (*Poecilia reticulata*), zebra fish (*Daneo rerio*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), golden orfe (*Leuciscus idus*), fathead minnow (*Pimephalis promelas*), silverside (*Menidia* spp), sheepshead minnow (*Cyprinodon variegatus*)	Lethality/survival, growth, development, hatching, deformation, biomarker measurements (vitellogenin, acetylcholinesterase, cellular energy)	DIN 38412; DIN 38415-6
			EPA/600/4-89/001; EPA/600/4-90/027; EPA/821/R-02/014
			EPS 1/RM/13; EPS 1/RM/28
			ISO 10229; ISO 7346-1,-2, -3; ISO 12890
			CSIR Reports NRE/WR/IR/2006/0015/C, NRE/WR/IR/2007/0032/C; WRC Reports 952/2/04, 1313/1/04
			Comp. Biochem. Physiol. 130C; *J. Aquat. Ecosystem Stress & Recovery* 6
Frog	Platanna (*Xenopus laevis*)	Lethality/survival, growth, development, hatching, deformation, metamorphosis, biomarker measurement (vitellogenin)	ASTM E1439-91
			WRC Report 358/1/98; Xema-SOP
			Ecotoxicol. Environ. Safety 53
Mollusc	Zebra mussel (*Dreissena polymorpha*), common mussel (*Elliptio complanata*), soft shell mussel (*Mya arenaria*)	Biomarker measurements (mixed function oxydases, methallothionein, DNA damage, lipid peroxidase, vitellogenin)	*Comp. Biochem. Physiol.* 128C; *Environ. Toxicol.* 14, 15, 17; *Environ. Toxicol. Water Qual.* 12
	Snail (*Melanoides tuberculata* – other species also listed)	Biomarker measurement (cellular energy)	*Environ. Pollut.* 129; *Environ. Toxicol.* Chem. 21
Echinoderm	Sea urchin (*Echinometra; Parachinus*; *Tripneustes*; *Stomoneuste*), purple urchin (*Abacia punctata*)	Fertilisation	EPA/821/R-02/014
			EPS 1/RM/27
Coelenterate	*Hydra attenuate*	Lethality, development	*Environ. Toxicol. Water Qual.* 12
Crustacean	Amphipod (*Rhepoxynius abronius* – other species also listed; *Grandierella* spp.; *Hyalella azteca*)	Lethality/survival, growth	EPS 1/RM/33; EPS 1/RM/35
Insect	Midge (*Chironomus tenans*; *C. riparius*)	Survival, growth	EPS 1/RM/32
Crustacean	Mysid (*Mysidopsis bahia*)	Lethality, growth, reproduction	EPA/600/4-90/027; EPA/821/R-02/014
Insect	Mayfly (*Ephemeroptera* spp.)	Lethality	Inst. Water Res., Rhodes University
Crustacean	Freshwater shrimp (*Caridina nilotica*)	Lethality	Inst. Water Res., Rhodes University
	Water flea (*Daphnia magna; D. pulex; Ceriodaphnia dubia*)	Lethality/survival, reproduction, biomarker measurements (galactosidase, cellular energy)	EPA/600/4-89/001; EPA/600/4-90/027
			EPS 1/RM/14; EPS 1/RM/21
			ISO 6341; ISO 10706
			OECD Guideline 202
			WRC Report 1313/1/04
			J. Aquat. Ecosystem Stress & Recovery 6
			Aquasurvey Inc.; Creasel Ltd.

TABLE 4.1: Summary of toxicity tests and methodologies selected for inclusion in the inventory of tests (continue)

Test organism/ material	Species	Measurements	Methodologies
Crustacean	Beaver-tailed fairy shrimp (*Thamnocephalus platyurus*)	Lethality	OECD Guideline 202
			Creasel Ltd.
	Brine shrimp (*Artemia salina*; *A. franciscana*)	Lethality	OECD Guideline 202
			Creasel Ltd.
Plant	Duckweed (*Lemna minor* Limnaeus)	Growth	EPS 1/RM/37
Alga	Barrel weed – red alga (*Champia parvula*)	Reproduction	EPA/821/R-02/014
	Green alga (*Selenastrum capricornutum; Scenedesmus subspicatus; Skeletonema costatum*; *Phaeodactylum tricornutum*)	Growth	EPA/600/4-89/001
			EPS 1/RM/25
			ISO 8692; ISO 10260; ISO 10253
			WRC Report 1313/1/04
			Environ. Toxicol. 15
			Creasel Ltd.
Protozoan	*Tetrahymena pyriformis*, *T. thermophila*	Growth, respiration	WRC Report 358/1/98; Umweltbundesamt Report No. UBA-FB 96-039
			Creasel Ltd.
Bacterium	*Pseudomonas putida*, *Vibrio fischeri*, *Salmonella typhimurium*, *Escherichia coli*	Growth, luminescence, mutation, enzyme activity (ß-galactosidase)	EPS 1/RM/42
			ISO 10712; ISO 11348-3; ISO 13829
			CSIR Report ENV-P-I 98184; WRC Reports 358/1/98, 1313/1/04
			J. AOAC Internat. 76; *Tox. Assess.* 2
			Environmental Biodection Products Inc.; Microbics Corp.
Fungus	Yeast (*Saccharomyces cerevisiae*)	Enzyme activity (ß-galactosidase)	WRC Report 1472/1/07
Animal cells	Fish liver, Golden hamster embryo, Chinese hamster cell line, Buffalo Green monkey kidney, human myelogenous leukemia, human blood	Viability, cell transformation, growth, inflammation, enzyme activity (dehydrogenase)	PhD Thesis University of Pretoria; WRC Reports 358/1/98, 1121/1/04
			Environ. Toxicol. 14*; Environ. Toxicol. Water Qual.* 12*; J Immunoassay* 21
Enzymes	Urease, acetylcholinesterase	Enzyme activity	WRC Report 358/1/98

4.2 Selecting and Defining the Toxicity Test Criteria to be Included in the Inventory of Tests

Two sets of toxicity test criteria were prepared for the inventory of tests. The first set of criteria supports the water quality management criteria that were identified for the water quality management contexts (paragraph 3.3). To simplify terminology, these toxicity test criteria are referred to as 'water quality management toxicity test criteria'. These criteria cover requirements in terms of the generic management criteria (Table 3.9), other management criteria (Table 3.10) and generic types of water sources. Table 4.2 summarises the water quality management toxicity test criteria.

TABLE 4.2: Water quality management toxicity test criteria

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 of 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 in Chapter 3, namely a plant kingdom and an 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 sample receipt in the laboratory until the test report is completed.
Other management criteria	Target kingdom (see 'protection' – 3.3.2.1)		The description is the same as the generic management criterion 'target kingdom' above.
	Optional criteria	Specificity	Whether or not the 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 the 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).

The second set of toxicity test criteria is supplementary to the first set of criteria (Table 4.2). The information captured for each of these criteria in the inventory of tests can be perused manually by an experienced user or biotoxicologist to make the final choice of appropriate tests. These criteria focus on general and technical aspects, as well as on appropriate uses of toxicity tests, and are intended to provide regulators, users and prospective toxicity testing laboratories with:

- a short overview of a test (*e.g.* test organism/material, test endpoint, etc.);
- a quick glimpse into laboratory requirements (*e.g*. maintenance, instruments, etc.);
- a comparison between test methodologies (*e.g.* small scale versus large scale testing, short-term exposure versus extended exposure, etc.); and
- an indication of the potential use (*e.g.* ecosystem integrity, domestic use, etc.).

The supplementary toxicity test criteria are summarised in Table 4.3.

TABLE 4.3: Supplementary toxicity test criteria

Criterion	Description
Appropriate uses	Whether or not the toxicity test can be applied for a specific water use, *e.g.* ecosystem integrity, domestic, etc.
Test method origin	Reference to the country or organisation that established and/or described the toxicity test methodology.
Type of exposure	The way in which the test organism/material is exposed to test samples, *e.g.* static, semi-static, etc.
Test organism/material	Living organism used for testing, or the type and origin of the biological material applied.
Test container	Size and type of container used for testing (provides an idea of the sample volume required).
Measurement endpoint	This refers to the long- and short-term biological responses or activities measured.
Measurement parameter	The measurement used to determine the biological response or activity, *e.g.* lethality – organism numbers; growth – dry weight; etc.
Exposure period	The time that the test organism/material is kept in contact with a test sample before the activity or response is measured.
Toxicity test endpoint	The presentation or expression of the measurement of the activity or response of a test organism/material after the designated exposure period, *e.g.* % lethality, % inhibition, etc.
% Detection limit	The concentration of a test sample where toxicity starts. This value is based on the variability of control results. When the variability between control results is low the detection limit of a test is low, *e.g.* 5%, and when the variability between control results is high the detection limit of a test is high, *e.g.* 25%.
Access to organism/material	Where the test organism/material can be sourced.
Organism/material maintenance	Provides an indication of the effort required to take care of the test organisms/material, *e.g.* routine maintenance, no maintenance, etc.
Special infrastructure/facilities	Refers to rooms and/or dedicated spaces for maintenance, storing and testing.
Dedicated instruments/equipment	Refers to special instruments or equipment required to conduct a toxicity test. (Every toxicity test facility should have basic instruments such as pH, oxygen and conductivity meters and thermometers to their disposal).
Date	Year of publication.
Authors	Names of person/s or organisation/s who published the methodologies.
Reference	Name of the journal/report/guideline or the contact details of institution where the methodologies can be found.

4.3 Compiling the Inventory of Tests

The inventory of tests was created in a spreadsheet, placing the toxicity tests on the vertical axis (first column of spreadsheet) under the heading 'Test name' and the toxicity test criteria on the horizontal axis (top row of spreadsheet). The required information for each test was captured in the cells of the matrix (Figure 4.1).

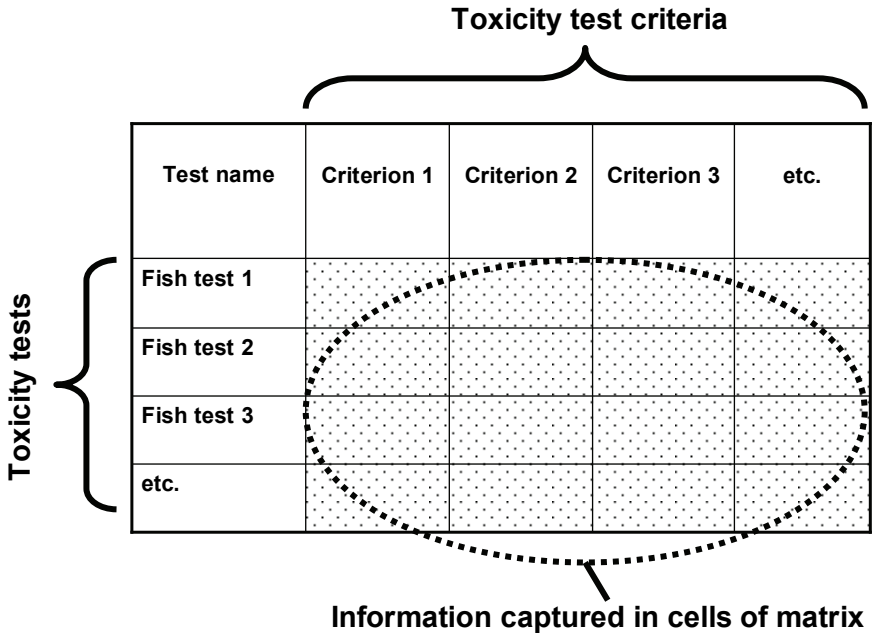

FIGURE 4.1: Placement of the toxicity tests, the toxicity test criteria and the required information in the spreadsheet

4.3.1 Toxicity tests

More than 100 toxicity tests are captured in the inventory. Fish, *Daphnia*, algal and bacterial tests formed the largest part of the test list. A considerable number of bivalve biomarker tests were also included in the list. To ensure some form of organisation, toxicity tests using similar organisms/material were grouped together. The test list starts with fish and frog tests, followed by invertebrate (mollusc, echinoderm, coelenterate, crustacean and insect) tests, plant and algal tests, microbial (protozoan, bacterium and fungal) tests, animal cell tests and *in vitro* enzyme tests (Table 4.1). As general rule the test names were kept as simple and concise as possible by referring only to the test organism/material (*e.g.* fish, frog, etc.) and the test measurement (*e.g.* lethality, growth inhibition, etc.). Where possible, the acute (*e.g.* lethal) tests were listed first, followed by the chronic (*e.g.* growth) tests within each group of organisms.

A number of test methodologies are similar or different tests have similar designations. It was, therefore, necessary to include additional information in the test name to distinguish between tests. In the case of fish tests the species name was added [*e.g.* fish (zebra) lethality; fish (rainbow trout) lethality]; the life stage was added if embryos, larvae, or juvenile fish are used instead of the traditional three month old fish [*e.g.* fish (fathead minnow) early

life stage lethality; fish (fathead minnow) larval survival and growth]; and the type of exposure was added where a specific type of exposure is applicable [*e.g.* fish (zebra) development (semi-static); whole fish (tilapia) acetylcholinesterase (active monitoring)]. In order to distinguish between similar bivalve tests the words 'freshwater' and 'marine' was added to the test name (*e.g.* freshwater bivalve methallothionein; marine bivalve methallothionein). 'Active monitoring' was added to tests applicable to in-stream testing as opposed to laboratory testing [*e.g.* freshwater bivalve methallothionein (active monitoring)]. To distinguish between the two sea urchin tests, the word 'purple' was added to the name of the appropriate test. The exposure period (*e.g.* 96-h and 10-d) was added to the mayfly- and shrimp lethality test names to distinguish between similar tests. In the case of the *Daphnia* tests the words '*pulex*' and '*magna*' were added to the test name if the specific species is used (*e.g. Daphnia pulex* lethality; *Daphnia magna* lethality). If the test is applicable to both species the word '*Daphnia*' is used [*e.g. Daphnia* galactosidase enzyme inhibition (IQ test kit); USA *Daphnia* lethality]. A further distinction was made between similar tests by adding the name of the country where the test method originates (*e.g.* Canada *Daphnia magna* lethality; USA *Daphnia* lethality). Where a test is available in a test kit form, this information was included in the test name for further distinction [*e.g. Daphnia* galactosidase enzyme inhibition (IQ test kit); *Daphnia pulex* immobilisation (DaphToxKit F^{TM}). Similar algal tests were distinguished by including the test container used in the test (*e.g.* algal 96-well microplate growth inhibition; algal 24-well microplate growth inhibition; algal scintillation vile growth inhibition); the way in which growth is measured [*e.g.* algal flask growth inhibition – chlorophyll measurement; algal flask growth inhibition – OD (optical density) measurement]; including the test kit name [*e.g.* algal growth inhibition (AlgalToxKit F^{TM})]; and by adding the word 'marine' (*e.g.* marine algal growth inhibition). In the case of the two protozoan growth inhibition tests a distinction was made by adding the test kit name to the applicable test. Bacterial tests with similar methodologies or similar designations were distinguished by adding the exposure period (*e.g.* 6-h bacterial growth inhibition; 16-h bacterial growth inhibition); by adding the word 'solid phase' in the case of use on sediments/soils [*e.g.* bacterial luminescence; bacterial luminescence (solid phase)]; and by adding the test kit name [bacterial (*Salmonella*) fluctuation; bacterial (*Salmonella*) fluctuation (Muta-chromoplate kit)].

The word 'whole' was added to test names where the measurement is carried out on the whole organism [*e.g.* whole fish (tilapia) acetylcholinesterase; whole snail cellular energy allocation (active monitoring)] instead of on a particular organ. In some instances the specific organ/material used for measurement was included in the test name (*e.g.* frog liver slice vitellogenin induction; fish primary hepatocyte viability). Where more than one organism and more than one test measure is used in one test this was also included in the test name [*e.g.* fish (rainbow and brook) lethality; *Ceriodaphnia* survival and reproduction]. The detailed toxicity test list can be found in the final guidance facility.

4.3.2 Toxicity test criteria

The toxicity test criteria were placed in the top row of the spreadsheet (see Figure 4.1). The water quality management toxicity test criteria were located next to the column listing the toxicity tests, following the same order as listed in Table 4.2. The supplementary toxicity test

criteria (Table 4.3) were placed after the water quality management toxicity test criteria. These criteria start with appropriate uses, *e.g.* 'Ecosystem integrity', 'Domestic', etc. The rest of the supplementary criteria were placed according to the list of criteria in Table 4.3.

4.3.3 Toxicity test information

4.3.3.1 Water quality management toxicity test criteria

4.3.3.1.1 Screening/Definitive

The majority of toxicity tests listed in the inventory of tests can be used for screening and definitive testing. The two types of application were accommodated in the inventory by listing the test name and the accompanying information in the cells of the spreadsheet in duplicate (duplicate rows), and designating the first row as 'screening' and the second row as 'definitive'. The information in the spreadsheet only differed for two of the criteria, namely 'Max day turnaround' (a longer period of time is required to finalise a test in the case of a definitive test) and the 'Toxicity test endpoint' [in the case of a screening test results are usually presented as a % lethality/effect/etc., while for a definitive test results are calculated or derived from a dose-response curve and provided as a lethal concentration (LC), or a no observable effect concentration (NOEC), etc.]. There were, however, a few exceptions: the turnaround time for the *Daphnia* IQ test, some rapid bacterial tests and the recombinant yeast screens; and the toxicity test endpoint of the zebra fish and frog liver slice vitellogenin induction tests, frog metamorphosis test, mutagenicity/genotoxicity tests and the yeast recombinant screens were the same for both types of application. Tests used for active monitoring (direct exposure to water in environment), *e.g.* whole fish (tilapia), bivalve and snail biomarker tests, as well as the amphipod and midge larval tests (direct exposure to sediment in laboratory), can only be applied as screening tests and were, therefore, only listed once (one row).

4.3.3.1.2 Legally defensible

The information was supplied as a 'Yes' or 'No'. Most of the tests listed in the inventory passed the requirement of being legally defensible on the basis that they are standard tests or are based on standard methodologies (*e.g.* lethality tests, growth and reproduction tests, development tests, etc.). Many of these tests have also been shown to be reproducible as evidenced by inter- and intra-laboratory and round-robin studies as well as have been published widely in scientific literature. The locally used *Daphnia* lethality and luminescent bacterial tests have been part of inter-laboratory testing since the early 2000s. The other locally developed/established tests considered to be legally defensible (fish lethality, *Daphnia pulex* survival and reproduction, algal growth inhibition, protozoan oxygen uptake, bacterial growth inhibition, bacterial mutagenicity, recombinant yeast estrogen screen, mammalian cell tests and *in vitro* enzyme tests) have a clear record of intra-laboratory evaluation and validation and methodologies and results have been published substantially in scientific literature and presented at national and international meetings. A large effort has also been made over the years to establish the methodologies in several local laboratories. It is expected that results of several of the international tests have been tested in courts of law.

During the late 1990s the Department of Water Affairs and Forestry (DWAF) has successfully settled a number of legal cases against industry based on the results of the locally established guppy and *Daphnia pulex* lethality tests and the algal and growth test. Tests not considered to be legally defensible, included the biomarker tests (except the local acetylcholinesterase and Canadian vitellogenin measurements), because they have not been applied widely and have not been used in inter-laboratory studies; the local frog embryo-larval survival and teratogenicity and the frog metamorphosis tests, because of limited use and problems with reproducibility; local mayfly and shrimp lethality tests, because they were only applied in one laboratory, on a non-continuous basis, and have not been exposed to inter-laboratory evaluation; and the locally used yeast androgen screen, because of problems with reproducibility.

4.3.3.1.3 Effect period

This criterion is designated as short- or long-term. From the toxicity test point of view the criterion does not only capture the period of observation time but also the effect/response measured.

All the lethality tests (scoring the number of surviving organisms) in the inventory of tests are designated as short-term (acute) tests, because the effect is observed within a short period of time compared to the life span of the organisms. Fish lethality tests are traditionally carried out over 96 h. Lethality can also be observed over a longer period of time and still be considered short-term (acute), *e.g.* 11 d in the case of the amphipod lethality test and 10 d in the case of the mayfly and freshwater shrimp tests. The period for lethality to manifest is shorter in the case of crustaceans such as *Daphnia*, fairy and brine shrimps and mysids and the coelenterate *Hydra* (24 to 48 h). A test is also considered as short-term (acute) when a sub-lethal response is measured over a short period of time (rapid test). The idea with these tests was to use a more sensitive parameter than lethality, but to reduce the observation time, to provide protection similar to that of the longer lethality tests. Microorganisms (*e.g.* protozoa, bacteria) with shorter life-spans and measurements such as oxygen uptake, enzyme activity, etc. were found to be particularly effective for this purpose. Typical sub-lethal tests designated as short-term tests include the sea urchin fertilisation tests (<2 h), *Champia* sexual reproduction test (48 h), *Daphnia* IQ test (1 h), protozoan oxygen uptake test (<1 h), protozoan ProToxKit (24 h), bacterial luminescence tests (<1 h), fish hepatocyte viability test (48 h), human cell tetrazolium reduction test (24 h) and the *in vitro* enzyme tests.

Toxicity tests based on sub-lethal effects that are manifesting over a long period of time as compared to the life span of the test organisms/material are designated as long-term (chronic). These include growth, development (including teratogenicity and metamorphosis), reproduction, mutagenicity/genotoxicity/carcinogenicity, endocrine disruption and enzyme and molecular tests. Growth tests range from hours to weeks (*e.g.* bacterial and protozoan: 6 to 48 h; algal and plant: 72 h to 7 d; insect and crustacean: 7 to 14 d; fish: 7 to 28 d). The shorter fish growth tests use larvae, which are more sensitive than adult fish. The mammalian cell colony formation test (growth) takes 7 d. Fish and frog development tests (examining early life stages, *e.g.* eggs, embryos, larvae) range from 2 to 30 d and 2 to 21 d, respectively. The shorter tests are highly sensitive, as specialised equipment such as

microscopes are used to continuously observe embryo changes and heartbeat. In the reproduction tests various life cycles of insects and crustaceans, from egg to adult, are observed. These observations take from 7 (mysid and *Ceriodaphnia*) to 21 d (*Daphnia*). Bacterial/mammalian cell mutagenicity/genotoxicity/carcinogenicity tests range from 4 h (sensitive enzyme measurement) to 7 d (multiplication of cells observed in terms of OD or colonies). Endocrine disruption observations can take from 3 to 10 d in the case of the recombinant yeast estrogen and androgen screens, and from 4 to 90 d in the case of vitellogenin measurements in fish, frogs and bivalves. Tests based on enzyme and molecular measurements (biomarker tests) in the laboratory range from 48 h (human blood cells) to 96 h (fish, bivalve and *Daphnia*). These tests take considerably longer when active monitoring is used (fish: 7 to 30 d; snails: 10 d, bivalves: 90 d).

Several long-term tests in the inventory involving growth and reproduction, also include lethality (= survival) measurements, *e.g.* fish larval survival and growth test; fish/frog embryo-larval survival and development test; amphipod survival and growth test; midge larval survival and growth test; mysid survival, growth and reproduction test; and several *Daphnia* survival and reproduction tests. In these instances, because of the longer observation period and/or the use of early life stage organisms, lethality is seen as a long-term measurement.

4.3.3.1.4 Target kingdom

Information for this criterion is captured in two columns (fourth and sixth columns) of the inventory of tests (generic management criterion and other management criterion – referred to as 'protection' in paragraph 3.2.2.1). The same information was supplied in both instances. The following rules were applied to decide if a toxicity test represents the plant kingdom or the animal kingdom:

Plant kingdom:	Only toxicity tests using plants.
Animal kingdom:	Tests using organisms/material from the animal kingdom, tests using organisms from the prokaryote kingdom, and tests using organisms genetically modified to model certain animal properties.

The majority of tests listed in the inventory of tests are designated as 'Animal'. These include short- and long-term tests employing fish, frogs and a wide range of invertebrates (*e.g.* molluscs, crustaceans, protozoa), as well as animal material such as whole organism homogenates [*e.g.* whole fish (tilapia) acetylcholinesterase], organs (*e.g.* frog liver slice vitellogenin induction), cells (*e.g.* fish primary hepatocyte viability, human blood cell inflammatory), and sub-cellular systems (*e.g.* marine bivalve methallothionein). The bacterial and yeast tests in the inventory are also designated as 'Animal'. Bacteria respond rapidly to adverse chemical activity because of their short life cycles. These tests are, therefore, considered to be very suitable for the protection of aquatic animals and man against acute and chronic chemical exposures. For example, the rapid bacterial luminescence and growth tests have proven to be indispensable as early warning systems in the case of emergency situations (*e.g.* industrial spills), while the bacterial mutagenicity/genotoxicity tests, that provide results within a few hours to days, have been used for many years to obtain

information on the potential carcinogenicity of chemicals in drinking as well as environmental waters. The yeast cells used in the recombinant yeast screens contain human estrogen and androgen receptors and have been genetically modified with the specific purpose to serve as rapid detectors of endocrine disrupting chemicals in water.

Only a few toxicity tests are designated as 'Plant'. The tests include an aquatic vascular plant (duckweed) growth test, several green algae growth tests, and a red algal (*Champia parvula*) reproduction test. The two *in vitro* enzyme assays (urease and acetylcholinesterase) are selectively sensitive to specific chemicals in water. They do not represent a particular organism kingdom but are considered to be tools that can protect any organism group against metal and pesticide pollution. They are thus designated as 'Any'.

4.3.3.1.5 Max days turnaround

The maximum turnaround time was established by revisiting every step during the execution of a test and recording the time taken by each activity from sample receipt to reporting the results. In short, these steps included sample and organism/material preparation, initiating the test, running the test (exposure period), scoring the results, processing the data, and writing and submitting the report. Where firsthand information was unavailable, test protocols were compared to similar methodologies for which the turnaround times were recorded, and times were allocated accordingly. The turnaround time is stated as full days. However, in a few instances the time is given as a fraction of a day (*e.g.* bacterial luminescence: 0.125 d; urease enzyme: 0.2 d).

For the majority of tests the exposure period forms the largest time component of the turnaround time. There are, however, a number of tests with a short exposure period compared to the turnaround time include: sea urchin fertilisation (<2 h compared to 24 h), *Hydra* lethality and development (24 h compared to 72 h), *Daphnia* IQ (1 h compared to 24 h), protozoan oxygen uptake (<2 h compared to 24 h), bacterial growth inhibition (6 h compared to 24 h, and 16 h compared to 48 h, respectively), bacterial luminescence (30 min compared to 3 h, and 20 min compared to 4 h, respectively), bacterial umu mutagenicity (4 h compared to 24 h), bacterial SOS chromotest (4 h compared to 48 h), mammalian cell transformation (7 d compared to 18 d), human blood cell inflammatory (18 h compared to 96 h), and the *in vitro* enzyme tests (30 min compared to 2 h, and 15 min compared to 3 h). In the case of fish, frog, and bivalve tests the test organisms are usually ready for use (organism preparation time short compared to the turnaround time). However, when fertilised eggs/embryos are required, as much as 18 h (overnight) have been added to the turnaround time for test material preparation. Most of the other tests require approximately 18 h for organism/material preparation. This involves stimulation of adult sea urchins to obtain sperm and eggs, and allowing *Hydra*, duckweed and *Champia* to grow to the required development stage. In the case of algal, protozoan, bacterial and yeast tests, overnight sub-cultures are prepared to have cells in the required growth stage. To obtain the required number of crustacean and insect offspring for testing, adult females with eggs in their brood chambers are usually sub-cultured during the afternoon before testing. The bacteria for the luminescent tests and the enzymes for the *in vitro* enzyme tests are ready for use (require only mixing with saline/buffer and a short incubation period). The preparation time for the fish primary

hepatocytes is about 24 h, and includes harvesting of liver cells and culturing the cells before exposure. It takes approximately 24- and 48 h, respectively to sub-culture the animal cells for the tetrazolium reduction- and colony formation tests. The mammalian cell transformation test requires multiple culturing and sub-culturing steps that can take up to 9 d. In the case of the human blood cell inflammatory test the preparation time includes finding suitable blood and culturing the cells to obtain the required stage before starting exposure.

Sample preparation usually takes from approximately 1 to 24 h and can include some of the following activities: pH, DO, temperature and conductivity measurements; filtration (in case of algal, protozoan, bacterial, yeast, animal cell, liver slice, and *in vitro* enzyme tests – to remove microbial contamination and particles); extraction and concentration (water or sediment/soil/material – applying mutagenicity, genotoxicity, carcinogenicity, estrogenicity and androgenicity tests or other tests that can be carried out on small sample volumes); and dilution (in case of definitive tests). The time added to the turnaround time for scoring the results (effect measurements) can range from approximately 1 h (*e.g.* lethality/survival/reproduction – organism numbers; growth – OD, cell numbers, front/cystocarp numbers; development – egg/embryo/larval numbers, enzyme and molecular measurements – OD, fluorescence; luminescence) to 1 d (*e.g.* growth – weight, length, appearance, number of colonies; development – microscopic examinations; cell transformation – number of transformed colonies). Although biomarker measurements in terms of OD and fluorescence are rapid, the preparation of the material before such measurements can also take up to one or two days.

The turnaround time of a definitive test is longer than that of a screening test. This is mainly because sample preparation is longer and because a range finding test (testing ten-fold dilutions) is usually carried out to establish the appropriate test concentration range. In the case of lethality/survival tests (*e.g.* fish, frog, *Hydra,* crustacean, insect), the definitive test is preceded by a lethality test to establish the effective concentration range (double the exposure time of a screening test). Long-term tests (*e.g.* growth inhibition, development and reproduction) that have long exposure periods (*e.g.* rainbow trout development: 30 d), are usually preceded by a lethality test to establish the test concentration range (exposure time: ≤96 h), while long-term tests that have exposure periods ≤8 d [*e.g.* whole fish (tilapia) acetylcholinesterase] and sub-lethal tests where lethal measurements are not possible (*e.g.* algal growth inhibition) are usually preceded by a range finding test that uses the same sub-lethal measurement as the definitive test.

4.3.3.1.6 Specificity

Specificity to a group of chemicals is designated by a Y (yes). No information was supplied when tests were non-specific (cells in matrix left blank). Specificity was stated for the following chemical groups: heavy metals, pesticides, other organics (*e.g.* PAHs), carcinogens, teratogens and endocrine disruptors.

The majority of tests are aimed at the general detection of toxic activity. However, a considerable number of tests have been developed to respond to specific chemical groups. For example, it is known that teratogens affect the early life stages of animals and cause

deformation/congenital effects. Those fish and frog tests listed in the inventory that observe embryo/larval development and teratogenicity were, therefore, developed with the specific purpose to detect teratogens in water. Carcinogens cause cancer in living organisms. Since cancer manifests over a long period of time, several tests have been established to detect this effect over a shorter period of time. The tests listed in the inventory of tests that are selectively sensitive to carcinogens include bacterial mutagenicity/genotoxicity tests (measuring genetic changes in terms of reverse mutations, enzyme activity and luminescence), a mammalian cell transformation assay (effects on genetic material observed as transformed colonies), and a few bivalve tests in which fluorescence measurements are used to detect DNA strand breaks. Endocrine disruption refers to the adverse effect of various natural and man-made chemicals on the endocrine (hormone) systems of humans and wildlife. Several tests in the inventory of tests have been developed to detect endocrine disrupting activity in water, namely zebra fish, frog, and bivalve vitellogenin (protein precursor of egg yolk) induction assays (selectively sensitive to estrogen and estrogen mimics), a frog metamorphosis test (selectively sensitive to chemicals affecting the thyroid function), and recombinant yeast tests (selectively sensitive to estrogen and androgen, and chemicals mimicking these hormones). Several enzyme-based assays in the inventory of tests are selectively sensitive to heavy metals, namely bivalve methallothionein and lipid peroxidase induction, and an *in vitro* urease inhibition test. In addition to its detection potential for endocrine disruptors, the frog metamorphosis test can also detect heavy metal pollution. Organophosphate and carbamate pesticides inhibit the activation of the enzyme acetylcholinesterase, a decrease in the breakdown of the enzyme substrate, therefore, serves as measure of the presence of these pesticides. The inventory of tests contains three whole fish (tilapia) acetylcholinesterase tests and an *in vitro* acetylcholinesterase test that is specific to organophosphate and carbamate pesticides. The frog metamorphosis test can also be used for the selective detection of pesticides. Certain enzymes have been found to be selectively sensitive to PAHs (and PCBs). These include mixed function oxidase and lipid peroxidase. A number of bivalve enzyme tests listed in the inventory are specific for PAHs (and PCBs).

4.3.3.1.7 Interferences

Sample properties such as very dark colour, high suspended solids, high salt content, oiliness and soapiness interfere with some of the toxicity tests listed in the inventory. Interferences are designated by a Y (yes). Blank cells in the matrix signify no interference. The information provided is based on the experience of the participants with the toxicity tests. Where firsthand information was not available, experiences with similar tests were used to make decisions.

Interferences are caused in some of the crustacean tests, algal tests, protozoan tests, bacterial tests, animal cell tests and *in vitro* enzyme tests. The tests that are affected and the interference caused is summarised in Table 4.4. Table 4.5 lists the tests that are not affected by the sample properties.

TABLE 4.4: Summary of toxicity tests affected by sample properties and the interferences caused

Sample property	Toxicity tests affected	Interference caused
Dark colour	*Daphnia*, *Ceriodaphnia*, *Thamnocephalus* and *Artemia* tests	These crustaceans are small and will not be visible by eye.[1]
	Algal growth inhibition tests	- Masks illumination – slowing down photosynthesis and growth. - Interferes with growth measurement – increases OD readings (colorimeter, turbidimeter, photometer, spectrophotometer, microplate reader).
	Protozoan and bacterial growth inhibition tests	Interferes with growth measurement – increases OD readings (colorimeter, photometer, spectrophotometer).
	Bacterial luminescence tests	Masks luminescence – low luminescence readings.
	Human cell tetrazolium reduction, human blood cell inflammatory and *in vitro* enzyme tests	Interferes with enzyme measurement – increases OD readings (microplate reader).
High suspended solids	*Daphnia pulex* and *Ceriodaphnia* tests	Organisms are small and are not easily distinguishable from particles. Particles can attach to appendices and inhibit movement.[2]
	Algal and bacterial growth inhibition tests in flasks/bottles	Large sample volumes are required for these tests. Membrane filtration (as used for other microbial tests) to remove particles and other microorganisms will be too time-consuming and expensive.
High salt content	Algal, protozoan and bacterial growth inhibition tests, human cell tetrazolium reduction, human blood cell inflammatory, and *in vitro* enzyme tests	A precipitate can form when concentrated growth medium (nutrients)/reagents are added to a sample containing high salts, interfering with growth/enzyme measurement – increases OD readings (colorimeter, turbidimeter, photometer, spectrophotometer, microplate reader).
	Animal cell and frog liver slice tests	Salts can form complexes with nutrients, rendering them unavailable for growth and metabolism.
Oiliness	*Daphnia*, *Ceriodaphnia*, *Thamnocephalus* and *Artemia* tests	Because these crustaceans are small, oil can attach to their bodies and appendices causing them to float – interfering with exposure. Respiration and movement can also be affected leading to death.
	Protozoan oxygen uptake test	Clogs membrane of oxygen probe, thus interfering with oxygen measurement.
	Bacterial (*Salmonella*) plate incorporation test	Oil separates from agar and masks/interferes with colony formation.
Soapiness	*Daphnia pulex, Ceriodaphnia, Thamnocephalus,* and *Artemia* tests	Reduces the refractive index of water, causing organisms to float – interfering with exposure.
	Protozoan oxygen uptake test	Causes bubbles and foam, interfering with oxygen measurement.

[1] If these are the only tests available, the intermediate observations could be omitted. Before final counting, the dark sample can be carefully poured off and replaced with clear control water.

[2] Upon standing particulate matter may settle in test containers, improving observation

4.3.3.1.8 Applicability

Applicability is designated by a Y (yes). Cells in the matrix were left blank when tests were not applicable. The information provided is based on the experience of the participants with the toxicity tests. Where firsthand information was not available, experiences with similar tests were used to make decisions.

Water resource type – All the toxicity tests in the inventory are applicable to inland water resources, except those using marine/estuarine organisms (*e.g.* sheepshead minnow and silverside (fish), marine bivalves, sea urchins, marine amphipod (*Rhepoxynius/ Grandierella*), mysid, *Artemia, Champia* and marine alga. Although *Vibrio fisherii* is a marine organism, the

TABLE 4.5: Summary of toxicity tests not affected by sample properties

Sample property	Toxicity tests not affected	Justification for decision
Dark colour	Fish, frog, mollusc, sea urchin, *Hydra*, amphipod, midge, mysid, mayfly, freshwater shrimp and duckweed tests	- Organisms/eggs/embryos/larvae/material sufficiently large to handle and see with naked eye. - Microscopes used to observe organism/embryo/larval development, teratogenicity and egg fertilization. - For biomarker measurements organisms are removed from water for further preparation/use.
	Daphnia IQ test	Organisms can be observed with naked eye because exposure is carried out in vials with a small diameter and organisms fluoresce.
	Champia reproduction test	- Large enough to handle and see with naked eye. - Placed in clean water for recovery after exposure.
	Protozoan oxygen uptake	Oxygen measurement not affected by colour.
	Bacterial plate incorporation-, fluctuation- and umu mutagenciity tests, bacterial SOS chromotest, recombinant yeast screens, and fish hepatocyte viability and liver slice tests	Sample extracts tested – colour removed.
	Mammalian cell transformation and colony formation tests	- Test samples removed after exposure. - Material stained and examined microscopically.
High suspended solids	Fish, frog, mollusc, sea urchin, *Hydra*, amphipod, midge, mysid, mayfly, freshwater shrimp, duckweed, *Champia*, *Daphnia* IQ, *Daphnia magna*, *Thamnocephalus*, and *Artemia* tests	- Organisms/eggs/embryos/larvae sufficiently large to distinguish from particles. - Microscopes used to observe organism/embryo/larval development, teratogenicity and egg fertilization. - For biomarker measurements and *Champia* reproduction test organisms are removed after exposure.
	Amphipod, midge, mysid and bacterial luminescent solid phase tests	Sediment tested directly – organisms will not be affected by particles.
	Algal (excluding flask tests), protozoan, bacterial luminescence, animal cell, frog liver slice and *in vitro* enzyme tests	Samples are membrane filtered to remove particles and to ensure sterility.
	Bacterial luminescence and mutagenicity/genotoxicity, animal cell, frog liver slice, recombinant yeast and *in vitro* enzyme tests	Samples are extracted, thus removing particles and ensuring sterility.
High salt content	Fish, frog, mollusc, sea urchin, *Hydra*, crustacean, insect, duckweed, *Champia*, protozoan oxygen uptake and bacterial luminescence tests	Exposure without medium addition – no precipitate formation.
	Algal growth tests using measurements other than OD	Precipitates will not interfere with growth measurement.
	Bacterial mutagenciity/genotoxicity tests and recombinant yeast screens	Salts removed during sample extraction – no interference with growth medium.
Oiliness	Fish, frog, mollusc, sea urchin, *Hydra*, crustacean [except *Daphnia* (excluding *Dahnia* IQ), *Ceriodaphnia*, *Thamnocephalus* and *Artemia*], insect, duckweed and *Champia* tests	Unlikely that oil will attach to such an extend to organisms and their appendices causing them to float or inhibiting their functions (excluding chemical effects).
	Algal, protozoan growth, bacterial (except plate incorporation), recombinant yeast, animal cell, frog liver slice and *in vitro* enzyme tests	Oiliness removed by sample filtration and/or extraction.
Soapiness	Fish, frog, mollusc, sea urchin, *Hydra*, crustacean (except *Daphnia pulex*, *Ceriodaphnia*, *Thamnocephalus* and *Artemia*), insect, duckweed and *Champia* tests	Unlikely that soapiness will cause organisms to float – interfering with exposure.
	Algal, protozoan growth, bacterial, recombinant yeast, animal cell, frog liver slice and *in vitro* enzyme tests	- Bubbles and foam will not cause organisms to float, thus interfering with exposure. - Will not interfere with measurements. - Will be removed by sample extraction.

luminescent bacterial tests are applicable to inland and estuarine resources. The frog liver slice test, bacterial mutagenicity/genotoxicity tests, and recombinant yeast screens (freshwater organisms) are also applicable to estuarine resources, because tests are carried out on extracted samples (salts removed).

Water resource zone – All the toxicity tests listed in the inventory are applicable to water bodies (*e.g.* river-, lake-, dam-, impoundment-, etc. water), except the amphipod and midge sediment tests and the bacterial luminescence solid phase test (tests specifically developed for sediment testing). Likewise, all the toxicity tests are applicable to groundwater, except the previously mentioned sediment tests, and the fish and bivalve active monitoring systems and the mayfly and freshwater shrimp artificial stream tests. Active monitoring is carried *in situ* (not possible for groundwater), while the artificial stream tests require too large volumes of water (large volumes of groundwater not always readily available). Apart from the previously mentioned direct sediment tests, a considerable number of the other listed toxicity tests can be applied to sediment supernatant/extracts/pore-water/interstitial water. These tests are listed in Table 4.6.

TABLE 4.6: Toxicity tests applicable to sediment testing

Toxicity test	Reason for selection
Early life stage fish lethality; zebra fish development; whole (zebra) fish acetylcholinesterase; frog embryo-larval survival and teratogenicity; frog embryo development; sea urchin and purple sea urchin fertilisation; mysid lethality; mysid survival, growth and reproduction; duckweed growth inhibition; *Champia* reproduction; algal flask growth inhibition protozoan growth inhibition	- Require relatively small sample volumes (approximately 50 to 500 mℓ). - Mostly static tests. - Sub-lethal tests conducted on diluted samples. - Applicable to supernatant and water extracts.
Hydra lethality and development; *Daphnia* IQ; *Daphnia* and *Ceriodaphnia l*ethality/immobilization; C*eriodaphnia* survival and reproduction; whole *Daphnia* energy allocation; *Thamnocephalus* lethality; *Artemia* immobilisation; algal 24-well microplate growth inhibition; algal scintillation vial growth inhibition; AlgalToxKit; bacterial growth inhibition; bacterial fluctuation	- Require small sample volumes (approximately 10 to 150 mℓ). - Mostly static tests. - Sub-lethal tests conducted on diluted samples. - Applicable to supernatant and water and organic solvent extracts.
Algal 96-well microplate growth inhibition; protozoan oxygen uptake; ProToxKit; bacterial luminescence; bacterial umu mutagenicity; bacterial Mutatox; bacterial SOS chromotest; fish primary hepatocyte viability; *in vitro* enzyme; frog liver slice vitellogenin induction	- Require small sample volumes (≤10 mℓ). - Applicable to supernatant, water and organic solvent extracts, pore-water and interstitial water.
Bacterial plate incorporation; recombinant yeast	- Require small sample volumes (≤10 mℓ). - Applicable to organic solvent extracts.

Mammalian and human cell tests are mainly aimed at human health protection and are, therefore, not applicable for sediment testing. Other fish and frog tests, laboratory based mollusc biomarker tests, *Daphnia* survival and reproduction tests and the mayfly and freshwater shrimp artificial stream tests are not used for sediment testing because the sample volumes required are too large. The fish and bivalve active monitoring systems are used for *in situ* water testing.

Water resource, discharge or extract water quality – The same tests apply to fresh- (low salinity) and brackish water (high salinity) as those listed for inland and estuarine resources, as discussed in the paragraph 'Water resource type'.

Solid or non-aqueous liquid – Most of the toxicity tests listed in the inventory of tests are applicable to the testing of leachates/run-off/extracts of stockpiles/solid waste/solid products used in water and wastewater treatment, as well as water/organic solvent chemical solutions/suspensions (used in treatment processes, *e.g.* paper industry; used to establish the sensitivity of developed tests; used as standard chemical solutions in inter-laboratory testing; etc.). These include tests requiring large sample volumes (*e.g.* some fish and frog tests, some mollusc tests, artificial stream tests) or small sample volumes (*e.g. in vitro* enzyme tests, animal cell tests, bacterial tests). Tests not applicable to solid or non-aqueous liquid, include fish, bivalve and snail tests used for active monitoring and direct sediment tests (amphipod and midge, and bacterial luminescence solid phase).

4.3.3.2 Supplementary toxicity test criteria

4.3.3.2.1 Appropriate uses

This criterion refers to five water uses: ecosystem integrity, domestic, irrigation, livestock and aquaculture. Toxicity tests that are applicable for a specific water use are designated by a Y (yes). Cells in the matrix were left blank when tests were not suitable. The information provided is based on the experience of the participants.

Ecosystem integrity – The water of concern in this instance should be safe to the organisms in the aquatic environment. All the toxicity tests listed in the inventory are suitable for this water use. Some of the tests will serve in the protection capacity, while others may have some predictive potential. The final battery of tests that will be selected for use will depend on the information required. The bacterial and yeast tests have been included in the list of tests suitable for this water use because they provide information on long-term effects, such as carcinogenicity and estrogenicity/androgenicity, respectively. The mammalian cell tests resemble the cellular systems of aquatic organisms and can provide answers on acute and chronic toxicity (carcinogenicity and inflammation). The *in vitro* enzyme tests are universal tests that can protect both plants and animals.

Domestic – This water use focuses on general domestic use and as drinking water source. Aquatic animals are often viewed as substitutes to protect human health. This is because of the similarity between the cellular and sub-cellular systems and functions of animals and man. Fish lethality tests, using a small size fish (guppy, golden orfe) or early life stage organisms (zebra fish, fathead minnow), all the *Daphnia* and *Ceriodaphnia* lethality tests, and the *Thamnocephalus* lethality test have been found suitable to protect human health because they can provide answers on acute chemical situations within a relatively short period of time (≤96 h). The two zebra fish and two frog development tests, examining embryo/larval development and teratogenicity, are applicable because they provide information on the presence of chemicals that can cause congenital effects in man. The *Hydra* development test is also listed because it provides rapid information (within 24 h) on potential long-term effects in man. Rapid tests using *Daphnia* (1 h IQ test) and protozoa (10 min oxygen uptake test) are applicable, because they provide rapid answers on acute toxicity in emergency situations. Several rapid bacterial tests have been established to protect man against acute toxicity, *e.g.* the 6 and 18 h growth inhibition tests and 30 min luminescence test. A number

of bacterial mutagenicity/genotoxicity tests are listed that are used for the detection of potential carcinogens (within hours to days) in domestic water. The frog liver slice, zebra fish metamorphosis, and the recombinant yeast tests are applicable to water for domestic use, because of their potential to detect endocrine disrupting chemicals in water. The mammalian and human cell tests have been listed, because they have been established with the specific purpose to protect human health against acute and chronic effects. As universal tests, the *in vitro* enzyme tests have been included as rapid tools, especially for emergency situations. Many of the listed tests have been successfully used over the past 30 years for drinking water testing (Kfir, 1981; Slabbert et al., 1998; Slabbert et al., 1999; Slabbert et al., 2007).

Irrigation – Water for this use should not contain chemicals that can affect consumers (humans and animals) and crops. All the tests that are applicable for domestic use (human health protection), are also applicable in this instance. To provide protection to plants, the duckweed and freshwater algal tests have been added to the list of tests applicable to this water use.

Livestock – The focus of protection in this instance is on livestock. All the tests that have been listed for domestic use are also applicable for livestock use. The justification being the similarities between human and other animal cellular and sub-cellular systems and biological functions.

Aquaculture – The quality of the water used for aquaculture should not adversely affect the animals and plants in these systems. Such systems are operated in fresh- and estuarine/marine environments. Most of the toxicity tests in the inventory have been found applicable for this water use. Many of the toxicity tests employ organisms that are used in aquaculture (*e.g.* tilapia, rainbow trout, *Xenopus,* bivalves, *Daphnia*, *Artemia*, algae) and should, therefore, be useful in a predictive capacity. Other tests have been selected because of their excellent protection potential (tests using exotic fish species, sea urchin tests, *Hydra* test, protozoan and bacterial tests, yeast tests, *in vitro* enzyme tests). The tests that are not applicable for this water use are captured in Table 4.7.

TABLE 4.7: Toxicity tests not applicable for the water use 'aquaculture'

Toxicity test	Reason for omission
Semi-static and flow-through zebra fish lethality	Zebra fish test included as protection tool. No need to include time-consuming tests if other fish tests can provide similar information.
Amphipod and midge sediment tests Solid phase bacterial luminescence test	- Other insect and crustacean tests (on water) listed. - Luminescent bacterial test on water more applicable.
Mayfly and freshwater shrimp artificial stream tests	Tests too time-consuming and require large volumes of water. Other invertebrate tests should be able to provide suitable information.
Protozoan growth inhibition tests	Protozoan oxygen uptake test preferred for rapid information.
Mammalian and human cell tests	Specific for human health protection.

4.3.3.2.2 Other supplementary criteria

Examples of the information captured in the inventory of tests for the other supplementary toxicity test criteria are presented in Table 4.8.

TABLE 4.8: Information captured for the supplementary toxicity test criteria (excluding appropriate uses)

Criterion	Information captured
Test method origin	Tests from the following countries are included in the inventory: South Africa, Canada, Germany, USA and Belgium. A large number of ISO methods are also listed.
Type of exposure	Fish tests are applied as static, semi-static or flow-through tests. Frog tests are static or semi-static. Bivalve biomarker tests can be applied as static, semi-static or flow-through tests, while active monitoring with fish and bivalves is carried out *in situ.* Test samples are re-circulated in artificial stream mayfly and freshwater shrimp tests. *Hydra* and sea urchin, as well as most of the insect and crustacean tests are static. Amphipod, midge and mysid tests can be static or semi-static, while the mysid lethality test can also be performed in flow-through form. *Daphnia* reproduction tests are usually semi-static. *Daphnia magna* is sufficiently large to also be applied as flow-through tests. The duckweed test is semi-static, while the *Champia*, algal, protozoan, bacterial, yeast, animal cell, and *in vitro* enzyme tests are all static tests.
Test organism/material	The genus and species names are given when living organisms are used for testing, *e.g. Danio rerio*, *Daphnia pulex, Escherichia coli*, etc. Where applicable, alternative species are also listed. In the case of cellular systems the type and origin of cells is given, *e.g.* primary Golden hamster embryo cells, human blood cells, etc. When test material at the sub-cellular level is used the material and origin is stated, *e.g.* urease enzyme from Jack beans.
Test container	The volume can range from minute, *e.g.* µℓ, to very large, *e.g.* >10 ℓ. In all cases the type of container and the container volume are given, *e.g.* 1 ℓ tank, 10 mℓ beaker, 24-well microplate (well volume: 3 mℓ), etc. If alternative containers can be used, these are also mentioned, *e.g.* dish, beaker or flask. When necessary, different container sizes are also stated, *e.g.* 150 to 500 mℓ beaker. In some instances the diameter and length of a container is mentioned, *e.g.* 60 mm Petri dish or 1 m artificial stream. When important (as in the case of endocrine disrupting compounds), the type of material is also given, *e.g.* glass. In the case of *in situ* (active monitoring) testing cages or strings can be used.
Measurement endpoint	Responses measured include lethality/survival/immobilisation, growth/growth rate, embryo/larval development, reproduction, DNA damage, respiration, luminescence, enzyme activity, etc. In some tests more than one endpoint is observed, *e.g.* organism survival, growth and egg development.
Measurement parameter	Some of the parameters used to measure endpoints include: Organism numbers in case of lethality; weight, length, cell numbers, optical density (OD), etc. in case of growth; fertilised and hatched egg numbers, embryo numbers, larval numbers, etc. in case of reproduction and development; OD, colony numbers, transformed colonies, etc. in case of DNA damage; OD and fluorescence in case of enzyme activity. In some instances more than one parameter is used for a measurement endpoint, *e.g.* algal growth can be determined in terms of chlorophyll, cell numbers or OD.
Exposure period	The exposure period in rapid tests can range from 10 min to a few hours, *e.g.* protozoan oxygen uptake: 10 min; bacterial growth: 6 h; human cell tetrazolium reduction: 24 h. Lethality tests using fish and larger invertebrates usually use a 96 h exposure period. The exposure periods of lethality tests employing smaller invertebrates, *e.g. Daphnia* and *Artemia* usually range from 24 to 48 h. Growth and reproduction tests using aquatic animals can range from 96 h to 30 d. Active monitoring (measuring various biomarkers) can use exposure periods as long as 90 d. Plant and algal growth tests usually range from 72 h to 7 d, while bacterial and protozoan growth tests range from 16 h to 48 h.

TABLE 4.8: Information captured for the supplementary toxicity test criteria (excluding appropriate uses) (continue)

Criterion	Information
Toxicity test endpoint	The result of a screening test and *in situ* testing is usually reported as a % effect, *e.g.* % lethality, % inhibition, % induction, etc. A calculated concentration value (using statistical analysis) is usually reported for a definitive test. Examples of such results include: LC10; LC50; EC20; EC50; IC25; IC50; etc. Some methods require reporting on a wide range of values, *e.g.* EC1; EC5; EC10; EC20 and EC50. In some definitive tests, results are also directly derived from the range of test concentrations used, *e.g.* NEC, NOEC, NOAEC (actual test concentration where no effect occurred) and LOEC (actual test concentration where the lowest effect occurred). In a number of tests (screening and definitive) test results are compared to control results to derive ratios, *e.g.* mutation ratio, induction ratio, etc.
% Detection limit	These values can range from 5 to 25%. Where more than one response is measured, *e.g.* lethality and deformation, a detection limit is given for each response. The test usually requires a statistical test of variance, and results are reported as significantly different or not from the control. When ratios are used for reporting results, a specific ratio is stated as the beginning of the adverse effect, *e.g.* MR≥2.0.
Access to organism/material	Most of the organisms/material used in local toxicity tests will be available in the form of standard cultures from local toxicity testing laboratories. Fish cultures can be established by obtaining fish from local fish farms, commercial fish suppliers, pet shops, and universities. Some of the fish species used in toxicity tests, *e.g.* fathead minnow, are not locally available. A permit will have to be required to import this species. Frogs are usually available from commercial suppliers and universities. Some of the invertebrates used in toxicity tests, *e.g.* bivalves, sea urchins, shrimps, mayflies, etc. can be collected from the field or obtained from aquaculture facilities and then reared in the laboratory, while others (*e.g. Hydra*, *Daphnia*, *Artemia*, midge, mysid, etc.) can be obtained as standard cultures from local or overseas toxicity testing laboratories. In some instances these cultures can also be obtained from universities. Some of the invertebrates, *e.g. Daphnia* and *Artemia,* are also available in their resting stages (ephippia) as part of test kits. Water plants such as duckweed and algae can be obtained *via* culture collections. Standard algal cultures are also available from local and international toxicity testing laboratories. Algae immobilised on beads can also be obtained from test kit suppliers. Protozoa, yeast and bacteria can be obtained from culture collections or toxicity testing laboratories. Some of these cultures are also available from suppliers of test kits (immobilised or lyophilised). Primary mammalian and fish cells are obtainable from animals reared in the laboratory. Cell lines are available from culture collections. Enzymes for *in vitro* testing can be obtained from chemical suppliers.
Organism/material maintenance	Many of the test organisms require routine maintenance, *e.g.* fish, frogs, larger invertebrates (bivalves, snails), smaller invertebrates (hydra, shrimps, *Daphnia*), duckweed, algae and protozoa. In the case of fish, frogs and larger invertebrates maintenance is tedious and involves regular cleaning of the holding containers, removal of debris, changing of water, measurement of water quality, and feeding. No maintenance will be necessary if organisms are used for testing immediately after field collection (*e.g.* sea urchins and may flies). The maintenance of smaller invertebrates, duckweed, algae and protozoa is less time-consuming and usually involves sub-culturing (once or twice per week) in fresh media (water with food or nutrients) to reduce the organism/cell numbers in stock cultures. Sterile conditions apply for duckweed, algal and protozoan culturing. If not used routinely, algal and protozoan cultures can be kept in the fridge for extended periods. No maintenance is required for invertebrates in resting form (ephippia) and organisms immobilised on beads (algae and protozoa). These are kept in the fridge until use. Bacterial cultures require the least maintenance as they are cultured on agar slopes that are kept in the fridge for extended periods. Many of the bacterial cultures are available in lyophilised form allowing them to be kept in the fridge, or the cultures can be frozen and kept in the freezer. Yeast and mammalian cell lines are also stored in frozen form until use. Enzymes are usually stored in the fridge or freezer.

TABLE 4.8: Information captured for the supplementary toxicity test criteria (excluding appropriate uses) (continue)

Criterion	Information
Special infrastructure/facilities	All the test organisms/material requires some form of temperature control for maintenance, storing and testing. Larger animals such as fish, frogs, bivalves, etc. are usually kept in aquatic rooms fitted with small or large tanks, aerators, water replacement systems and illumination. Smaller temperature controlled spaces (*e.g.* corner of a laboratory) are required for smaller invertebrates (*Hydra*, shrimps, *Daphnia*), duckweed and algae. Commercial incubators are usually used to culture protozoa, yeast, bacteria and cell cultures/tissue. As a rule separate culturing and testing facilities are required to avoid contamination of stock cultures. In most instances illumination is optional during testing. However, light control is necessary for duckweed and algae, and during the hatching process of invertebrate resting stages. Other special infrastructure for maintaining cultures/material and for conducting toxicity tests include cold storage/freezers (lyophilised and frozen organisms/cells and enzymes as well as reagents) and sterile rooms with laminar flow cabinets.
Dedicated instruments/equipment	In general, lethality tests do not require any special instruments or equipment. Dosing and mixing equipment are used in some semi-static and flow-through tests employing fish and larger invertebrates. Microscopes of various complexity are required to examine fish, frog and urchin egg/embryo/larval development, to count and examine smaller invertebrates (*e.g. Hydra*, *Artemia*), and to examine and count algae and fish/mammalian cells and cell colonies. A counting chamber, called a haemacytometer, is usually used with a microscope for cell counting. Microplate readers (miniature spectrophotometers) are usually used in tests where enzyme/molecular responses (colour changes) are recorded (fish, frog and invertebrate biomarkers, recombinant yeast tests, bacterial mutagenicity/genotoxicity tests, fish/human cell tests and the *in vitro* urease test). Microplate shakers are often required for mixing in microplate studies. Micro-fluorometers are used for some of the enzyme/molecular measurements (mixed function oxydases, DNA damage, lipid peroxidase, hepatocyte viability). A variety of photometers (measurement of OD) are required to determine algal, protozoan, yeast and bacterial growth, *e.g.* microplate reader, spectrophotometer, turbidimeter and colorimeter. Algal growth can also be determined in terms of chlorophyll, using a fluorometer, or as cells with a particle counter. A colony counter is used to examine bacterial colony formation (bacterial plate incorporation assay). The luminescent bacterial tests are carried out with specially designed luminometers with incubation baths, and the protozoan oxygen uptake assay uses a dedicated oxygen monitoring system with accompanying water bath. Other requirements include an irradiation facility/system for the irradiation of mammalian cells (transformation assay), a fluorescent light box to examine fluorescing *Daphnia* in the rapid IQ test, and an oven to determine the dry weight of organisms in some of the growth tests.
Date	No more than two references are listed, due to limited space in the inventory. In the case of two references, the first date will be designated by 'a)' and the second by 'b)'.
Authors	Only the first and second author is mentioned. In the case of more than two authors, the first author and '*et al*.' is noted. In the absence of authors, organisations and environmental protection agencies are listed. In the case of two references, the first author/s or organisation or environmental protection agency will be designated by 'a)' and the second by 'b)'.
Reference	The report name and number or journal name and number or the company name/contact details are provided. Where possible abbreviations were used. In the case of two references, the first reference will be designated by 'a)' and the second by 'b)'. A complete list of references is included as Appendix B.

5. INTEGRATION OF INFORMATION

This task entailed integrating the list of available toxicity tests (chapter 4) with the specific management criteria associated with each management context (chapter 3). Figure 5.1 illustrates the principle.

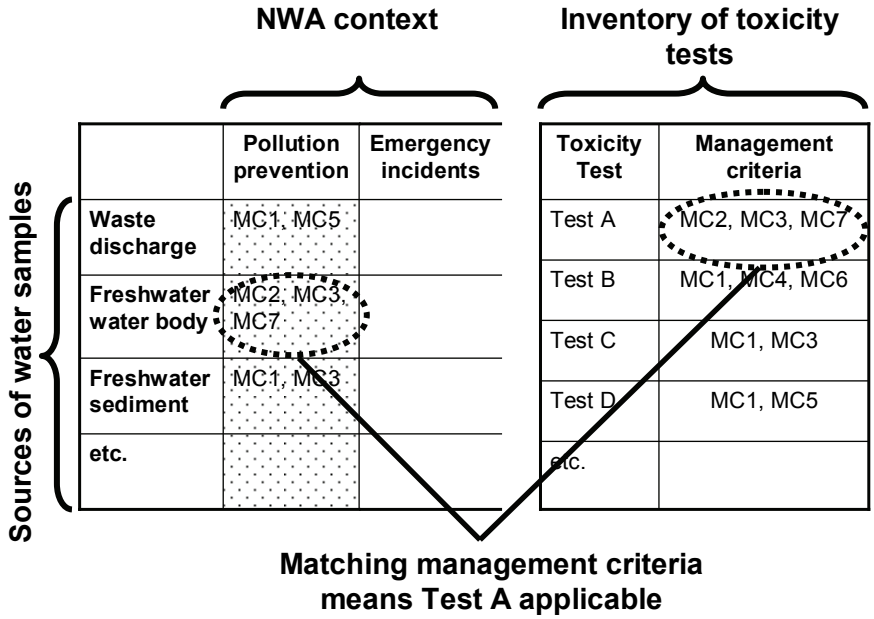

FIGURE 5.1: Illustration of matching toxicity tests with management contexts

For all the management criteria, the matching process is carried out automatically. How the matching is done is explicit in the rules applied to establish the management criteria in National Water Act (NWA) contexts and in the way the information is captured for individual tests according to these criteria. This has the following advantages:

- The matching rules are entirely transparent.
- Maintenance and updating of the guidance facility interface to incorporate new developments in future is greatly simplified.

The following general approach to matching is taken.

- *Compulsory requirements*. An exact match between all the compulsory management requirements is enforced. The user has no control over these other than by defining the NWA context, water resource type, zone, inland water resource quality (or the discharge or extract water quality – only source context) and the source of toxicants – only source context. These criteria are defined in the management criteria matrices (Table 3.11 and 3.12).

- *Situation-specific requirements matched automatically.* The user may also specify certain other requirements that only serve to shorten the list of toxicity tests based on

the management criteria. Specifying these requirements is not mandatory. These include:

- a requirement in terms of 'protection' where the user can choose the kingdom of organisms to be afforded protection. By choosing a kingdom, the classification of the management criteria matrix is overruled. The only classification that cannot be overruled is the kingdom 'Animal' for the context 'reserve determination' for basic human needs. The choice of kingdom can be based on the intended water use (Table 3.2) or the nature of the water source (Table 3.3); and

- optional requirements focusing on the presence or absence of specific toxic chemical groups in a sample (*e.g*. heavy metals, etc.) and sample properties that can cause interferences in tests (*e.g*. very dark colour, etc.)

- *Situation-specific requirements matched by user.* For some requirements (supplementary information), it is not appropriate to attempt automatic matching. In these cases, the guiding principles are provided for the user's consideration but not enforced in the spreadsheet interface. The user (or biotoxicologist), therefore, decides which of the recommended tests are appropriate.

After the above general approach to matching has been completed the following requirements might still require clarification (involving further refinement of the recommended list of tests).

- Selection of toxicity tests that protect specific organism groups. *The guide has been developed with a more lenient protective perspective and is broadly based on target groups at the level of kingdoms (animal and plant). In some instances the user might want to consider the relationship between a test and target organism at the target group or target organism level. Should a situation like this occur, where the predictive context is more appropriate, the user needs to exercise much greater caution in the choice of toxicity tests. In particular, there must be a well-established relationship between the test organism and target organism (if they are not identical).*
- Distinguishing between direct, pore water and water extract sediment toxicity tests. *The automatic selection of toxicity tests only takes place at the broad 'sediment' level. In some instances it might be necessary to choose specific sediment tests.*
- Specific requirements in terms of cost and turnaround time.
- Tests only applicable to organic solvent extracts.
- Tests only used as definitive tests.

6. GUIDANCE FACILITY

Because of the multi-dimensional nature of the information (chapters 3 and 4), and because the management contexts and inventory of tests were compiled in spreadsheets, the most appropriate means to produce the guide was in spreadsheet format. The spreadsheet facility is intended to provide preliminary guidance on the most appropriate toxicity tests to use in the National Water Act (NWA) contexts related to water resources (only) or discharge sources and associated water resources.

6.1 Structure

There are 13 worksheets in the guidance facility. These are designated: 'BACKGROUND', 'INSTRUCTIONS', 'RESOURCE CONTEXT', 'RESOURCE TOX. TESTS', 'SOURCE CONTEXT', 'SOURCE TOX. TESTS FOR DISCHARGE', 'SOURCE TOX. TESTS FOR RESOURCE', 'Resource Management Criteria', 'Source Management Criteria', 'Resource MM', 'Source MM Discharge', 'Source MM Resource' and 'Inventory of tests', and are captured in the order listed, from left to right in the interface.

The 'BACKGROUND' worksheet contains the title of the guidance facility and refers to the project title and number and project members. It also provides a short objective and a disclaimer. It also indicates that the 'Instructions' worksheet should be consulted for detailed instructions on how to proceed.

The 'INSTRUCTIONS' worksheet contains all the information required to use the spreadsheet facility. Included are a five step summary of the instructions, an interpretation of the list of recommended toxicity tests and detailed information and instructions. The detailed information and instruction section addresses the 'water resource context' and the 'discharge source context'. For each of these contexts, definitions and instructions are given with reference to the specific NWA contexts, water resource type, the zone, water resource water quality, protection and optional criteria. Links are also provided to go to the 'RESOURCE CONTEXT' and the 'SOURCE CONTEXT' worksheets to specify the requirements. Additional information can be obtained in Chapter 3.

The worksheets 'RESOURCE CONTEXT' and 'SOURCE. CONTEXT' (sheets 3 and 5) list the specific NWA contexts, the water resource type, the zone, water resource water quality, protection and optional criteria. Two additional management criteria are included in the 'SOURCE CONTEXT' worksheet, namely 'Source of toxicants' and 'Discharge or Extract Water Quality'. As an example, the information contained in the 'RESOURCE CONTEXT' worksheet is shown in Figure 6.1. On the left hand side of the list of contexts and criteria, is a column where the user can state the specific requirement (*i.e.* 1 for the required NWA context, water resource type and the zone; fresh or brack for the water resource water quality; any, animal or plant for protection and Y or N for optional criteria). Further left of the stated requirement column is a list of rules (*i.e.* A, B, G, H, K, X and Z). These rules are applicable to the contexts and criteria on the right hand side of the worksheet and are explained at the bottom of the sheet. The specific requirements that can be stated for

'Source of toxicants' in the 'SOURCE CONTEXT' worksheet are '1' or 'blank' (*i.e.* leave blank) and for 'Discharge or Extract Water Quality' 'Fresh' or 'Brack'.

TABLE 6.1: Example of the information contained in the 'RESOURCE CONTEXT' worksheet

Water Resources

Water Resource Management

Rule	State your requirement	***National Water Act context***
A	**1**	*Classification & Resource Quality Objectives*
A		*Reserve - Basic Human Needs*
A		*Reserve - Ecological*
A		*Monitoring ecosystem health*
A		*Monitoring compliance with Resource Quality Objectives*
A		*National status & trends monitoring*
		Water Resource type
A	**1**	*Inland water resource*
A&H		*Estuarine*
		Zone
A	**1**	*Water body*
A		*Sediment*
A&G		*Groundwater*
		Water Resource Water Quality
B	**Fresh**	*Is the Inland Water Resource fresh or brackish? (Fresh, Brack)*
		Protection
K	**Plant**	*What kingdom of organisms do you wish to afford protection? (Any, Animal, Plant)*
		Optional criteria that will avoid inappropriate tests being performed
Y	**N**	*Are you sure no heavy metals are present? (Y=you are sure/N=not sure)*
Y	**N**	*Are you sure no pesticides are present? (Y=you are sure/N=not sure)*
Y	**N**	*Are you sure no other organics are present? (Y=you are sure/N=not sure)*
Y	**N**	*Are you sure no carcinogens are present? (Y=you are sure/N=not sure)*
Y	**N**	*Are you sure no teratogens are present? (Y=you are sure/N=not sure)*
Y	**N**	*Are you sure no endocrine disruptors are present? (Y=you are sure/N=not sure)*
Z	**N**	*Is the sample very dark in colour? (Y/N)*
Z	**N**	*Does the sample have very high levels of suspended solids? (Y/N)*
Z	**N**	*Does the sample have a very high salt content? (Y/N)*
Z	**N**	*Is the sample oily? (Y/N)*
Z	**N**	*Is the sample soapy? (Y/N)*

Rule	***Rule applied when establishing the applicability of a test***
A	Only tests that satisfy the criteria in the Management Criteria Matrix will be recommended.
H	If estuarine, only tests applicable to brackish waters will be recommended.
B	If fresh or brack is specified, only tests applicable to these will be recommended (Inland Water Resource only)
G	*In situ* tests for groundwater are excluded.
K	Only tests on a system appropriately associated with the target kingdom will be recommended. *(This will only be effective if the target kingdom specified in the Management Criteria Matrix is "Any".)*
Y	If sure, then toxicity tests that are specific to these compounds are excluded from the recommended list.
Z	If so, toxicity tests sensitive to this interference are excluded from the recommended list.

Links exist between the 'RESOURCE CONTEXT' and 'SOURCE CONTEXT' worksheets and the 'INSTRUCTION' worksheet to enable the user to quickly verify information and definitions.

The 'RESOURCE TOX. TESTS', 'SOURCE TOX. TESTS FOR DISCHARGE' and the 'SOURCE TOX. TESTS FOR RESOURCE' worksheets (sheets 4, 6 and 7) display all the information captured in the inventory of tests (list of tests in first column, criteria as headings of the following columns, and information inside the cells of the sheet). Detailed information on the inventory of tests can be found in Chapter 4. As an example, Figure 6.2 shows some of the information that is captured in the 'RESOURCE TOX. TESTS' sheet (the other two sheets are similar). The top line of the sheet contains the following headings (from left to right): 'National Water Act Context', 'Organisms', 'Specificity', 'Interferences', 'Applicability' and 'Supplementary Information'. The second line shows the requirements that are specified in the worksheets 'RESOURCE CONTEXTS' and "SOURCE CONTEXTS' for 'National Water Act Context', 'Organisms', 'Specificity', 'Interferences' and 'Applicability'. Between the column listing the toxicity tests and the criteria columns is a column named 'Recommended'. To the left of the heading are the instructions on how to select the recommended tests (marked with a ✓). It should be noted that the user should scroll up and down the rows, and from left to right across the columns to observe all the information.

Tables 3.11 and 3.12 are captured in the guidance facility as the worksheets 'Resource Management Criteria' and 'Source Management Criteria'. The 'Resource MM', 'Source MM discharge' and 'Source MM resource' worksheets (MM = 'Match Matrix') are matrices that contain the list of toxicity tests and all the management criteria (from 'screening/definitive' to 'solid or non-aqueous liquid'). These matrices store marks that indicate whether individual criteria for each toxicity test satisfy the specified user requirements (=1) or not (=0). The last worksheet 'Inventory of toxicity tests' is the master toxicity test list as prepared in Chapter 4.

The last six worksheets (mentioned in the previous paragraph) do not require any inputs or usage from the user. These worksheets are contained in the spreadsheet facility, but they are hidden to avoid misunderstanding and accidental changes.

The technical detail and updating of the spreadsheet facility is summarised in Appendix C.

6.2 Use of the Guidance Facility

The use of the facility is demonstrated by means of a typical request for toxicity testing. After the recommended list of tests generated by the facility is shown and discussed, slight modifications are made to the typical request. The facility is then applied again to view and discuss the recommended tests.

6.2.1 Typical request for toxicity testing

The water quality manager/water user is concerned about the water quality in an **inland river** after a **fish kill** occurred. There is no observable run-off or discharge in the vicinity of the fish kill. Which toxicity tests can be performed on the river water?

TABLE 6.2: Example of some of the information contained in the worksheet 'RESOURCE TOX. TESTS'

Water Resource Management

Toxicity test guide for the resource		National Water Act context					Organisms	Specificity			
Requirements based on specified context:		**Screening**	**Any**	**Any**	**Any**	**30**	**Plant**				
STEP 1: *TO DISPLAY ALL TESTS: Click on Autofilter icon and select All.* ***STEP 2:*** *TO DISPLAY ONLY RECOMMENDED TESTS, click on Autofilter icon. If YES appears in drop-down menu, select YES. If YES does not appear, there are no tests that satisfy your requirements.* *Use horizontal scroll bar to see supplementary information.*	***Recommended***	***Screening /Definitive***	***Legally defen-sible***	***Effect period***	***Target kingdom***	***Max days turnaround***	***Target kingdom***	***Heavy metals***	***Pesticides***	***Other organics (e.g. PAHs)***	***Carcinogens***
Test name											
Fish (tilapia) early life stage lethality		Screening	Yes	Short-term	Animal	5	Animal				
Fish (tilapia) early life stage lethality		Definitive	Yes	Short-term	Animal	11	Animal				
Fish (guppy) early life stage lethality		Screening	Yes	Short-term	Animal	5	Animal				
Fish (guppy) early life stage lethality		Definitive	Yes	Short-term	Animal	11	Animal				
Fish (zebra) lethality		Screening	Yes	Short-term	Animal	5	Animal				
Fish (zebra) lethality		Definitive	Yes	Short-term	Animal	11	Animal				
Fish (zebra) early life stage lethality		Screening	Yes	Short-term	Animal	5	Animal				
Fish (zebra) early life stage lethality		Definitive	Yes	Short-term	Animal	11	Animal				
Fish (rainbow trout) lethality		Screening	Yes	Short-term	Animal	5	Animal				
Fish (rainbow trout) lethality		Definitive	Yes	Short-term	Animal	11	Animal				
Fish (rainbow and brook trout) lethality		Screening	Yes	Short-term	Animal	5	Animal				
Fish (rainbow and brook trout) lethality		Definitive	Yes	Short-term	Animal	11	Animal				
Fish (golden orfe) lethality		Screening	Yes	Short-term	Animal	5	Animal				
Fish (golden orfe) lethality		Definitive	Yes	Short-term	Animal	11	Animal				
Fish (zebra) lethality (semi-static)		Screening	Yes	Short-term	Animal	5	Animal				
Fish (zebra) lethality (semi-static)		Definitive	Yes	Short-term	Animal	11	Animal				
Fish (zebra) lethality (flow through)		Screening	Yes	Short-term	Animal	5	Animal				

6.2.1.1 Selecting the appropriate toxicity tests

Step 1. A river is a water resource. Select the 'Resource Context' worksheet.

Step 2. The fish kill indicates that the aquatic environment is at risk. The specific NWA context in this case is the 'Reserve – Ecological'. Select this context by placing a 1 in the column 'State your requirement' next to the 'Reserve – Ecological. There is already a 1 next to one of the specific contexts, delete this 1 (only one context can be marked).

Step 3. Next, the 'Water Resource type' should be stated. The river is an inland water resource (not 'Estuarine'). Therefore, place a 1 in the column 'State your requirement' next to 'Inland water resource'. Ensure that only 'Inland water resource' is marked.

Step 4. This step involves a decision about the 'Zone'. The concern is the 'Water body' (not 'Groundwater' or 'Sediment'). State the requirement by marking the 'Water body'. Again, ensure that only 'Water body' is marked.

Step 5. This step involves a decision about the 'Water Resource Water Quality'. The river is an inland resource. The requirement that needs to be stated is, therefore, 'Fresh' (not 'Brack').

Step 6. Next, the requirement in terms of 'Protection' should be stated. Because fish died, it is expected that other aquatic animals as well as plants could be affected. Therefore, rather state 'Any' so that tests using both plants and animals are included in the recommended list of tests.

Step 7. Now, requirements in terms of 'Optional criteria' should be stated. No information was provided on the presence or absence of specific chemical groups (*e.g*. heavy metals, pesticides, etc.). Likewise, no properties were highlighted that could cause interferences with tests (*e.g.* very dark colour, oiliness, etc.). Because you are not sure about the presence or absence of specific chemical groups, the requirement 'N' should be marked (not 'Y'). Likewise, use the requirement 'N' for the sample properties (not 'Y'). By not marking the 'Y', the list of recommended tests will not be shortened.

Step 8. Having stated all the requirements, the next step is to select the worksheet 'RESOURCE TOX. TESTS'.

Step 9. The recommended tests are marked with a 'Yes'. In this worksheet, click on the autofilter icon (in the 'Recommended' column) and select 'All'. This action will ensure that all tests are displayed. Then, click on the autofilter once more and select 'Yes'. The shortlist of recommended tests are now captured.

6.2.1.2 Discussion of the list of recommended tests

On the second line of the worksheet, notice the requirements based on the specified context ('Reserve – Ecological'), namely 'Screening', 'Yes' (for legally defensible), 'Any' (for effect

period), ‘Any’ (for target kingdom) and 30 (for max days turnaround). These were determined by the criteria classifications in the hidden ‘Resource Management Criteria’ worksheet (also captured in Table 3.11). With reference to ‘Organisms’, ‘Any’ is stated (choice of organisms you wish to afford protection). No selection is shown for ‘Specificity’ and ‘Interferences’. With reference to ‘Applicability’, a ‘Y’ is recorded for ‘Inland water resource’, ‘Water body’ and ‘Fresh’.

Because very general requirements were stated, this list of recommended tests is very long (60 tests) and include a variety of test organisms/material (fish, frog, bivalve, *Hydra*, crustaceans, duckweed, algae, protozoa, bacteria, yeast, animal cells and enzymes). All the tests are screening tests (direct testing) and are legally defensible. The effect period of the tests is short- or long-term (lethality and sub-lethal responses), the organisms represent the target kingdom animal and plant, and it takes from a few hours to 29 d to obtain results (this is not considered an emergency situation, therefore, tests that take a longer period of time are included). Some of the tests that are included, are selectively sensitive to specific chemical groups, *e.g.* heavy metals, pesticides, teratogens and endocrine disruptors. Such tests could assist in pinpointing the source of pollution. If positive responses are obtained for pesticides or endocrine disruptors, the cause of the fish kill could be attributed to agricultural run-off. Tests showing positive for heavy metals, could suggest metal pollution from an industry upstream of the fish kill. The list of tests indicate which tests can be carried out, not which must be carried out. In consultation with a biotoxicologist, and using the supplementary information supplied for each test, select the most appropriate battery of tests to perform from the recommended list.

6.2.3 Modifications to the typical request for toxicity testing

6.2.3.1 Modification 1

If ‘Animal’, instead of ‘Any’, was stated in step 6 (paragraph 6.2.1.1), no plant tests would have been included in the recommended list of tests (recommended list of tests now 52).

6.2.3.2 Modification 2

If the fish kill is an emergency situation (toxicity tests should provide quick answers), the ‘Source Context’ instead of the ‘Resource Context’ worksheet should be selected. When in this sheet:

- State ‘Emergency Incidents’ as the specific NWA context;\
- State ‘Inland water resource’ as the ‘Water Resource type’
- State ‘Water body’ as the ‘Zone’;
- State ‘Fresh’ as the ‘Water Resource Water Quality’
- Leave ‘Source of toxicants’ blank;
- State ‘Fresh’ as the ‘Discharge or Extract Water Quality’;
- State ‘Any’ as ‘Protection’; and
- State ‘N’ for the ‘Optional Criteria’.

Having stated all the requirements:

- Select the worksheet ‘SOURCE TOX. TESTS FOR RESOURCE’. In this worksheet, click on the autofilter icon (in the ‘Recommended’ column) and select ‘All’. Then, click on the autofilter once more and select ‘Yes’. The recommended tests are now captured.

On the second line of the worksheet, notice the requirements based on the specified context (‘Emergency Incidents’), namely ‘Screening’, ‘Any’ (for legally defensible), ‘Any’ (for effect period), ‘Any’ (for target kingdom) and 1 (for max days turnaround). With reference to ‘Organisms’, ‘Any’ is stated. No selection is shown for ‘Specificity’ and ‘Interferences’. With reference to ‘Applicability’, a ‘Y’ is recorded for ‘Inland water resource’, ‘Water body’ and ‘Fresh’.

Only seven tests are listed (*Daphnia* IQ, protozoan oxygen uptake, bacterial growth inhibition, bacterial luminescence, bacterial mutagenicity, *in vitro* urease and *in vitro ac*etylcholinesterase). These are all rapid tests. Some of the tests are selectively sensitive to heavy metals, pesticides and carcinogens. If these tests are carried out they could indicate where the pollution came from.

6.2.3.3 Modification 3

If the fish kill is an emergency situation (modification 2), and the source of pollution is known, *e.g.* an industry upstream of the fish kill, then go through all the steps followed for modification 2, but instead of selecting ‘SOURCE TOX. TESTS FOR RESOURCE’, select ‘SOURCE TOX. TESTS FOR DISCHARGE’. Again, click on the autofilter icon (in the ‘Recommended’ column) and select ‘All’. Then, click on the autofilter once more and select ‘Yes’. The recommended tests are now captured.

On the second line of the worksheet, notice the requirements based on the specified context (‘Emergency Incidents’), namely ‘Any’ (for screening/definitive), ‘Any’ (for legally defensible), ‘Any’ (for effect period), ‘Any’ (for target kingdom) and 1 (for max days turnaround). With reference to ‘Organisms’, ‘Any’ is stated. No selection is shown for ‘Specificity’ and ‘Interferences’. With reference to ‘Applicability’, a ‘Y’ is recorded for ‘Fresh’ (not for ‘Inland water resource’ or ‘Water body’ as is the case for the ‘SOURCE TOX. TESTS FOR RESOURCE’).

In this instance, eight tests are recommended. Because ‘Any’ is stated for ‘Screening/Definitive’, some of these eight tests will be applicable as both screening and definitive tests if the turnaround time is ≤ 1 d. All the tests are the same as those recommended for the ‘SOURCE TOX. TESTS FOR RESOURCE’, except that the solid phase luminescent bacterial test is also included in the list. This is a sediment test that is usually omitted from tests on the water body. It has been included for discharge testing because the only applicability rule that is applied is ‘Y’ for ‘Fresh’ (see previous paragraph). A biotoxicologist would usually omit this test, because the luminescent bacterial test on water is already included.

6.3 General Observations Regarding the Use of the Source Context Worksheet

When specifying requirements in the 'SOURCE CONTEXT' worksheet, the recommended list of toxicity tests can be viewed in the 'SOURCE TOX. TESTS FOR DISCHARGE' and 'SOURCE TOX. TESTS FOR RESOURCE'. The idea with the two separate worksheets was that in the case of the 'SOURCE TOX. TESTS FOR DISCHARGE', only tests for the discharge will be recommended. This list of tests does not take any resource requirements into account. This approach is applicable when a person only wants to know if a particular discharge is toxic and how toxic it is. The approach is very useful in the case of pilot plants where treatment processes are evaluated and decisions are required on the suitability of effluents for discharge. The more frequent use of the 'SOURCE CONTEXT' worksheet will be to protect the aquatic environment that will be receiving the discharge. In this instance the toxicity tests recommended for the resource would be the appropriate tests to use. The recommended list will include only screening tests. For testing the discharge, the corresponding definitive tests will be applicable.

When evaluating the facility, it was found that the 'SOURCE TOX. TESTS FOR DISCHARGE' and the 'SOURCE TOX. TESTS FOR RESOURCE' were the same when the specified 'Water Resource type' and the 'Discharge or Extract Water Quality' were the same, *e.g.* 'Inland water resource' and 'Fresh' or 'Estuarine' and 'Brack'. When the 'Water Resource type' is specified as 'Inland water resource' and the 'Discharge or Extract Water Quality' is specified as 'Brack', then freshwater toxicity tests will be listed for the water resource, and saltwater toxicity tests will be listed for the discharge (or *vice versa*). An important rule to remember is that when the resource needs to be protected, always use tests from the list of tests recommended for the resource.

7. CONCLUSIONS AND RECOMMENDATIONS

- The project team succeeded in meeting the objective of the study, namely to establish a guide for the selection of toxicity tests that support the information requirements of the National Water Act (NWA).

- An interactive and well-structured process enabled the identification of an appropriate suite of management criteria that could serve as the critical link between the NWA management contexts and the inventory of tests.

- Inputs from local as well as international scientists assisted in the production of an inventory of tests that contains a wide range of well-established toxicity tests with sufficient information associated with each test to satisfy the information requirements of the NWA management contexts.

- Because of the multi-dimensional nature of the information requirements, the guide was produced as an electronic spreadsheet facility (in Excel).

- The production of a spreadsheet facility, instead of a paper document, ensured that the guide is available in a compact, user-friendly, format.

- There is no complex interfacing involved in the spreadsheet facility. The guide can, therefore, be effortlessly accessed and understood by a wide range of users, including water resource and source managers, water users, researchers and educators.

- The spreadsheet facility has been developed in a highly modular way and contains a number of worksheets. This does not only improve objectivity, but also makes updating of the facility relatively straightforward.

- The built-in filtering capabilities of the spreadsheet allow the automatic matching of information in different worksheets. Successive filtering can also produce shorter information lists.

- The management criteria of the various management contexts are automatically matched with the available list of toxicity tests to produce a list of toxicity tests appropriate to each management context. This has the advantage that objectivity is imposed on the choice of tests.

- Automatic matching is not possible for all the requirements (*e.g.* supplementary information). This provides the user the opportunity to reduce the list of tests that was automatically generated to suite his/her specific requirements. The specific requirements may include:

    - ❖ Toxicity tests that protect specific organism groups;

- ❖ Toxicity tests for direct, pore water and water extract sediment testing;
- ❖ Tests that are reasonably priced;
- ❖ Tests that have a certain turnaround time;
- ❖ Tests only applicable to organic solvent extracts; and
- ❖ Tests only used as definitive tests.

- The toxicity tests recommended by the guidance facility are tests that **can** be carried out, not **must** be carried out.

- The inventory of tests includes local as well as international tests. The recommended list of tests will, therefore, also display a mixture of these groups of tests. Some of the local tests are carried out routinely in local toxicity test laboratories. The user's requirements should be discussed with a biotoxicologist. If required a recommendation on a reputable international testing laboratory can be made by the biotoxicologist.

- The inventory of tests includes a wide range of tests that has the potential to address a variety of eventualities. It was, however, not possible to include every possible toxicity test. Furthermore, in some instances only a limited number of tests have been developed that use specific test organisms, *e.g.* aquatic plants, or only a limited number of tests are applicable to certain waters, *e.g.* estuarine water. It is, therefore, possible that a situation may arise where the user selects a combination of management contexts and criteria that could result in a very limited list of recommended toxicity tests, or no toxicity tests are recommended. In such a case the user's requirements should be discussed with a biotoxicologist.

- An important function of the guide is to identify gaps with reference to specific tests for specific information requirements. If such gaps are identified, it should be followed up with the Department of Water Affairs and Forestry or the Water Research Commission (WRC), to ensure that tests for that specific purpose are developed.

- The functioning of the guidance facility has been evaluated extensively. It is, however, possible that a specific combination of management contexts and criteria do not provide a satisfactory list of tests or the expected list of tests. In such a situation the matter should be addressed with a biotoxicologist, or the custodian/developers of the guidance facility can be contacted.

- It is expected that the guidance facility will be a valuable educational tool. It is hoped that it would stimulate interest amongst biological students to follow a career in aquatic toxicology and that it would sensitise water resource and source managers to the importance of toxicity testing in water quality management.

- It is recommended that the WRC acts as custodian of the guidance facility. This entails that any updating or expansion of the facility is arranged and guided by the WRC. The latest version of the facility will, therefore, be available from the WRC.

8. REFERENCES

BLAISE C and FÉRARD J-F (2005). *Small-scale freshwater toxicity investigations, Volume 1:Toxicity test methods.* Springer, Dordrecht, The Netherlands, 551 pp.

DWAF (1997). White Paper on a National Water Policy for South Africa. Department of Water Affairs and Forestry, Pretoria.

DWAF (1998). South African Water Quality Guidelines. Volumes 1-7, Second Edition. Department of Water Affairs and Forestry, Pretoria.

HURTER E, POOL EJ and VAN WYK JH (2002). Validation of an *ex vivo Xenopus* liver slice bioassay for environmental estrogens and estrogen mimics. *Ecotoxicol. Environ. Safety* **53** (1) 178-187.

JOOSTE S (2000). Aspects of aquatic ecotoxicity in water resource management: An input to the WRC Workshop on Ecotoxicology. 10 May 2000, Pretoria.

PERSOONE G, JANSSEN and DE COEN W (2000). *New microbiotests for routine toxicity screening and biomonitoring.* Kluwer Academic/Plenum Publishers, New York, 550 pp.

POOL EJ, VAN WYK JH and LESLIE AJ (2000). Inflammatory activity as an indicator of water quality: The use of human whole blood cultures. *J. Immunoassay*. **21** (4) 387-399.

REPUBLIC OF SOUTH AFRICA (1996). *Bill of Rights*, In: The Constitution of the Republic of South Africa. Act No. 108, Chapter 2, Section 24.

REPUBLIC OF SOUTH AFRICA (1998). National Environmental Management Act. Act No. 107, 19 November 1998.

REPUBLIC OF SOUTH AFRICA (1998). National Water Act. Act No. 36, 20 August 1998.

SCHERMAN P-A and PALMER CG (2000). A protocol for acute toxicity testing using selected riverine invertebrates in artificial stream systems. Version 1.0. DWAF Report produced by the Institute for Water Research, Rhodes University, Grahamstown.

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

SLABBERT JL and VENTER EA (1999). Biological assays for aquatic toxicity testing. *Wat. Sci. Tech.* **39** 367-373.

SLABBERT JL, OOSTHUIZEN J, VENTER EA, HILL E, DU PREEZ M and PRETORIUS PJ (1998a). Development of guidelines for toxicity bioassaying of drinking and environmental waters in South Africa. WRC Report No. 358/1/98, Pretoria.

SLABBERT JL, OOSTHUIZEN J, VENTER EA, HILL E, DU PREEZ M and PRETORIUS PJ (1998b). Development of procedures to assess whole effluent toxicity. WRC Report No. 453/1/98, Pretoria.

SLABBERT JL, VENTER EA, CARLSSON FHH, ELLERBECK C and PIENAAR E (1999). Establishment of methodologies for oestrogen mimicking substances and genotoxicants. CSIR Report ENV-P-I 98184, Pretoria.

VENTER EA, SLABBERT JL, JOUBERT A, VORSTER A and BARNHOORN I (2004). Biomarker assays for the detection of sub-lethal toxicity in fish: Operational Manual. WRC Report No. 952/2/04, Pretoria.

WELLS PG, LEE K and BLAISE C (1998). *Microscale testing in aquatic toxicology – advances, techniques and practice.* CRC Press, Boca Raton, Florida, USA, 679 pp.

WHITCUTT JM, EMMETT RA, TSEKI R, MBATHA Z and VAN HEERDEN P (2004). An integrated approach to biomonitoring of waste water for the presence of biologically active agents. WRC Report No. 1121/1/04, Pretoria.

APPENDIX A

CHAPTERS, PARTS AND SECTIONS OF THE NATIONAL WATER ACT THAT WERE EXAMINED TO IDENTIFY INFORMATION REQUIREMENTS FOR TOXICITY TESTS (relevant sections underlined)

INDEX

CHAPTER 3: PROTECTION OF WATER RESOURCES

Part 1: Classification system for water resources

12. Prescription of classification system

Part 2: Classification of water resources and resource quality objectives

13. Determination of class of water resources and resource quality objectives
14. Preliminary determination of class or resource quality objectives
15. Giving effect to determination of class of water resource and resource quality objectives

Part 3: The Reserve

16. Determination of Reserve
17. Preliminary determinations of Reserve
18. Giving effect to Reserve

Part 4: Pollution prevention

19. Prevention and remedying effects of pollution

Part 5: Emergency incidents

20. Control of emergency incidents

CHAPTER 4: USE OF WATER

Part 1: General principles

21. Water use
22. Permissible water use
23. Determination of quantity of water which may be allocated by responsible authority
24. Licences for use of water found underground on property of another person
25. Transfer of water use authorisations
26. Regulations on the use of water

Part 2: Considerations, conditions and essential requirements of general authorisations and licences

27. Considerations for issue of general authorisations and licences
28. Essential requirements of licences
29. Conditions for issue of general authorisations and licences
30. Security by applicant
31. Issue of licence no guarantee of supply

APPENDIX B

COMPREHENSIVE LIST OF REFERENCES FOR THE TOXICITY TESTS CAPTURED IN THE GUIDANCE FACILITY

AQUA SURVEY INC (1997). *Daphnia* galactosidase enzyme inhibition (IQ) test. Aquasurvey@aol.co.

BANTLE JA and SABOURIN TD (1991). Standard guide for conducting the frog embryo teratogenesis assay – *Xenopus* (FETAX). ASTM E1439-91, American Society for Testing and Materials, Philadelphia, 1-11.

BLAISE C, GAGNÉ F, PELLERIN J and HANSEN P-D (1999). Measurement of vitellogenin-like properties in the hemolymph of *Mya arenaria* (Saguenay Fjord, Canada): a potential biomarker for endocrine disruption. *Environ. Toxicol.* **14** (5) 455-465.

BLAISE C, FORGET G and TROTTIER S (2000). Toxicity screening of aqueous samples using a cost-effective 72-h exposure *Selenastrum capricornutum* assay. *Environ. Toxicol.* **15** 352-359.

BLAISE C, GAGNÉ F, PELLERIN J, HANSEN P-D and TROTTIER S (2002). Molluscan shellfish biomarker study of the Quebec, Canada, Saguenay Fjord with the softshell clam *Mya arenaria. Environ. Toxicol.* **17** 170-186.

DE COEN WM and JANSSEN CR (1997). The use of biomarkers in *Daphnia magna* toxicity testing. 4. Cellular energy allocation: A new methodology to assess the energy budget of toxicant-stressed *Daphnia* populations. *J. Ecosystem Stress & Recovery* **6** 43-55.

DEUTSCHES INSTITUT FUR NORMUNG (1989). German standard methods for the examination of water, waste water and sludge – bioassays (group L). Determining the tolerance of fish to the toxicity of waste water by way of a dilution series (L31). Part 31. DIN 38412-31, Berlin.

DEUTSCHES INSTITUT FUR NORMUNG (2001). German standard methods for the examination of water, waste water and sludge – Subanimal testing (group T). Determination of the non-acute-poisonous effect of waste water to fish eggs by dilution limits (T6). Part 6. DIN 38415-6, Berlin.

ENVIRONMENTAL BIODETECTION PRODUCTS INC (2003). Bacterial Muta-chromoplate test. Tel: 905 794 2325; Fax: 905 794 233.

ENVIRONMENTAL BIODETECTION PRODUCTS INC (2003). Bacterial SOS chromotest. Tel: 905 794 2325; Fax: 905 794 233.

ENVIRONMENT CANADA (1992). Fertilization assay using Echnoids (Sea Urchins and Sand Dollars). EPS 1/RM/27, Ottawa.

ENVIRONMENT CANADA (1997). Test for reproduction and survival using the cladoceran, *Ceriodaphnia dubia.* Second Edition. EPS 1/RM/21, Ottawa.

ENVIRONMENT CANADA (1997). Growth inhibition test using the freshwater alga *Selenastrum capricornutum*. Second Edition. EPS/RM/25, Ottawa.

ENVIRONMENT CANADA (1997). Test for survival and growth in sediment using the larvae of freshwater midges (*Chironomus tentans* or *Chironomus riparius*). EPS 1/RM/32, Ottawa.

ENVIRONMENT CANADA (1997). Test for survival and growth in sediment using the freshwater amphipod *Hyalella azteca.* EPS 1/RM/33, Ottawa.

ENVIRONMENT CANADA (1998). Toxicity tests using early life stages of salmonid fish (rainbow trout). Second Edition. EPS 1/RM/28, Ottawa.

ENVIRONMENT CANADA (1998). Reference method for determining acute lethality of sediment to marine or estuarine amphipods. EPS 1/RM/35, Ottawa.

ENVIRONMENT CANADA (1999). Test for measuring the inhibition of growth using the freshwater macrophyte, *Lemna minor*. EPS 1/RM/37, Ottawa.

ENVIRONMENT CANADA (2000). Reference method for determining acute lethality of effluents to rainbow trout. Second Edition. EPS 1/RM/13, Ottawa.

ENVIRONMENT CANADA (2000). Reference method for determining acute lethality of effluents to *Daphnia magna.* Second Edition. EPS 1/RM/14, Ottawa.

ENVIRONMENT CANADA (2002). Reference method for determining the toxicity of sediment using luminescent bacteria in a solid-phase test. EPS 1/RM/42, Ottawa.

FISH F, LAMPERT I, HALACHIMI A, RIESENFELD G and HERZBERG M (187). The SOS Chromotest kit: A rapid method for the detection of genotoxicity. *Tox. Assess.* **2** 135-147.

GAGNÉ F and BLAISE C (1997). Validation of the rainbow trout hepatocyte model for ecotoxicity testing of industrial wastewater. *Environ. Toxicol. Water Qual.* **12** 305-314.

GAGNÉ F and BLAISE C (2000). Organic alkali-phosphates in biological materials: A generic assay to detect vitellogenin in biological tissues. Technical methodology. *Environ. Toxicol.* **15** 243-247.

GAGNÉ F, BLAISE C, VANAGGELEN G, BOIVIN P, CHONG-KIT R, JONCZYK E, MARION M, KENNEDY S, LEGAULT R and GOUDREAULT J (1999). *Environ. Toxicol.* **14** 429-437.

GAGNÉ F, BLAISE C, LACHACNE B, SUNAHARA GI and SABIK H (2001). Evaluation of estrogenic effects of municipal effluents to the freshwater mussel *Ellipto complanata. Comp. Biochem. Physiol.* **128C** 213-225.

GAGNÉ F, BLAISE C, AOYAMA I, LUO R, GAGNON C, COUILLARD Y, CAMPBELL P AND SALAZAR M (2002). Biomarker study of a municipal effluent dispersion plume in two species of freshwater mussels. *Environ. Toxicol.* **17** 149-159.

HOLBECH H, ANDERSEN L, PETERSEN GI, KORSGAARD B, PEDERSEN KL and BJERREGAARD P (2001). Development of an ELISA for vitellogenin in whole body homogenate of zebrafish (*Danio rerio*). *Comp. Biochem. Physiol.* **130C** (1) 119-131.

HURTER E, POOL EJ and VAN WYK JH (2002). Validation of an *ex vivo Xenopus* liver slice bioassay for environmental estrogens and estrogen mimics. *Ecotoxicol. Environ. Safety* **53** (1) 178-187.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1989). Water quality – Fresh water algal growth inhibition test with *Scenedesmus subspicatus* and *Selenastrum capricornutum*. ISO 8692, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1992). Water quality – Measurement of biochemical parameters: Spectrometric determination of the chlorophyll-a concentration. ISO 10260, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1994). Water quality – Determination of the prolonged toxicity of substances to freshwater fish [*Oncorhynchus mykiss* Walbaum (Teleostei, Salmonidae)]. ISO 10229, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1995). Water quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. ISO 10253, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1995). Water quality – *Pseudomonas putida* growth inhibition test (*Pseudomonas* cell multiplication inhibition test). ISO 10712, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1996). Water quality – Determination of the inhibition of the mobility of *Daphnia magna* Straus (Clodecera, Crustacea) - Acute toxicity test. ISO 6341, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (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 7346-1, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1996). Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)]. Part 2: Semi-static method. ISO 7346-2, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1996). Water quality – Determination of the acute lethal toxicity of substances to a freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)]. Part 3: Flow-through method. ISO 7346-3, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1998). 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 11348-3, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (1999). Water quality – Determination of toxicity to embryos and larvae of freshwater fish [*Brachydanio rerio* Hamilton-Buchanan (Teleostei, Cyprinidae)]. Semi-static method. ISO 12890, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (2000). Water quality – Determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea). ISO 10706, Geneva.

INTERNATIONAL ORGANISATION FOR STANDARDISATION (2000). Water quality – Determination of the genotoxicity of water and waste water using the umu-test. ISO 13829, Geneva.

KFIR R (1981). The detection and assay of potential carcinogens and toxicants in water by tissue culture techniques. Thesis for the Degree of Doctor of Science in the Faculty of Science, University of Pretoria.

KLOAS W, OPITZ R and LUTZ I (2003). Xema – Standard Operation Procedure. Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin.

MICROBICS CORPORATION (1993). Bacterial Mutatox™ test. Standard Operational Procedure. Carlsbad, USA, Tel: 800 642 7629; Fax: 800 528 5558.

OECD (1984). *Daphnia* sp. acute immobilisation test – Part I. OECD Guideline for testing of chemicals, No. 202, Organisation for Economic Cooperation and Development, Paris.

OECD (1984). Algal growth inhibition test. OECD Guideline for testing of chemicals, No. 201, Organisation for Economic Cooperation and Development, Paris.

PAULI W and BERGER S (1996). Proceedings of the International Workshop on a Protozoan Test Protocol with *Tetrahymena* in aquatic toxicity testing. Umweltbundesamt Report UBA-FB 96-039, Berlin.

PERSOONE, G (1990). ArToxKit M™ – *Artemia* toxicity screening test for estuarine and marine water. Standard Operational Procedure. Creasel Ltd, Deinze, Belgium.

PERSOONE, G (1995). ThamnoToxKit F™ – Crustacean toxicity screening test for freshwater. Standard Operational Procedure. Creasel Ltd, Deinze, Belgium.

PERSOONE, G (1996). AlgalToxKit F™ – Freshwater toxicity test with microalgae. Standard Operational Procedure. Creasel Ltd, Deinze, Belgium.

PERSOONE, G (1996). DaphToxKit F^{TM} *magna* – Crustacean toxicity screening test for freshwater. Standard Operational Procedure. Creasel Ltd, Deinze, Belgium.

PERSOONE, G (1996). DaphToxKit F^{TM} *pulex* – Crustacean toxicity screening test for freshwater. Standard Operational Procedure. Creasel Ltd, Deinze, Belgium.

PERSOONE, G (1998). ProToxKit F^{TM} – Freshwater toxicity test with a ciliate protozoan. Standard Operational Procedure. Creasel Ltd, Deinze, Belgium.

POOL EJ, VAN WYK JH and LESLIE AJ (2000). Inflammatory activity as an indicator of water quality: The use of human whole blood cultures. *J. Immunoassay*. **21** (4) 387-399.

SCHERMAN P-A and PALMER CG (2000). A protocol for acute toxicity testing using selected riverine invertebrates in artificial stream systems. Version 1.0. DWAF Report produced by the Institute for Water Research, Rhodes University, Grahamstown.

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

SLABBERT JL, OOSTHUIZEN J, VENTER EA, HILL E, DU PREEZ M and PRETORIUS PJ (1998). Development of guidelines for toxicity bioassaying of drinking and environmental waters in South Africa. WRC Report No. 358/1/98, Pretoria.

SLABBERT JL, VENTER EA, CARLSSON FHH, ELLERBECK C and PIENAAR E (1999). Establishment of methodologies for oestrogen mimicking substances and genotoxicants. Environmentek, CSIR Report ENV-P-I 98184, Pretoria.

SLABBERT JL, MOLETSANE M, VENTER EA, FAKUDE L and MOTSEPE A (2006). Short- and long-term zebrafish toxicity tests and statistical programmes for data analysis. CSIR/NRE/WR/IR/2006/0015/C, Pretoria.

SLABBERT L, VENTER A, MOLETSANE M and FAKUDE L (2007). Short- and long-term toxicity tests and statistical programmes for data analyses (zebrafish and *Daphnia pulex*). CSIR/NRE/WR/IR/2007/0032/C, Pretoria.

SLABBERT JL, VENTER EA, MOTSEPE A and MOLETSANE M (2007). Method development for biochemical procedures related to estrogen and androgen screening of water and sediment samples: 2002-2003. Biological/biochemical methods and the establishment of *in vivo* techniques for the detection of endocrine disruptors in water systems: 2003-2005. *In* Burger, A.E.C. (ed.), 2007. *Implementation of a Research Programme for Investigating Endocrine Disrupting Contaminants in South African Water Systems.* Vol. 2, Appendixes 3A+B. WRC Report No. 1402/1/07.

SMOLDERS R, BERVOETS L and BLUST R (2002). Transplanted zebra mussels (*Dreissena polymorpha*) as active biomonitors in an effluent dominated river. *Environ. Toxicol. Chem.* **21** 1889-1896.

SMOLDERS R, BERVOETS L, DE COEN W & BLUST, R. 2004. Cellular energy allocation in zebra mussels exposed along a pollution gradient: Linking cellular effects to higher levels of biological organisation in an effluent dominated river. *Environ. Pollut.* **129** 99-112.

SUN TSC and STAHR HM (1993). Evaluation and application of a bioluminescent bacterial genotoxicity test. *J AOAC Internat*. **76** (4) 893-898.

TROTTIER S, BLAISE C, KUSUI T and JOHNSON EM (1997). Acute toxicity assessment of aqueous samples using a microplate-based *Hydra attenuata* assay. Technical methodology. *Environ. Toxicol. Water Qual.* **12** 265-271.

US EPA (1989). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Second Edition. EPA/600/4-89/001, Office of Research and Development, US Environmental Protection Agency, Cincinnati.

US EPA (1991). Methods for measuring the acute toxicity of effluents to aquatic organisms. Fourth Edition. EPA/600/4-90-027, Office of Research and Development, US Environmental Protection Agency, Cincinnati.

US EPA (2002). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Third Edition. EPA-821-R-02-014, US Environmental Protection Agency, Washington.

VENTER EA, SLABBERT JL, JOUBERT A, VORSTER A and BARNHOORN I (2004). Biomarker assays for the detection of sub-lethal toxicity in fish: Operational Manual. WRC Report No. 952/2/04, Pretoria.

WHITCUTT JM, EMMETT RA, TSEKI R, MBATHA Z and VAN HEERDEN P (2004). An integrated approach to biomonitoring of waste water for the presence of biologically active agents. WRC Report No. 1121/1/04, Pretoria.

APPENDIX C

TECHNICAL DETAIL AND UPDATING OF THE SPREADSHEET FACILITY

Technical detail:

Worksheet name	May be modified by
BACKGROUND	Developer only
INSTRUCTIONS	Developer only
RESOURCE CONTEXT	User
RESOURCE TOX. TESTS	Developer only[1]
SOURCE CONTEXT	User
SOURCE TOX. TESTS FOR DISCHARGE	Developer only[1]
SOURCE TOX. TESTS FOR RESOURCE	Developer only[1]
Resource Management Criteria	Developer only
Source Management Criteria	Developer only
Resource MM	Developer only
Source MM Discharge	Developer only
Source MM Resource	Developer only
Inventory of tests	Developer only[1]

[1] User can use the autofilter to restrict display to only the recommended tests (those marked with a 'Yes'). However, the user may not modify any cell contents.

Updating of the facility:

The facility has been developed in a highly modular way. This not only improves objectivity but also makes updating of the facility relatively straightforward. The following table summarises how the facility should be updated.

To …	Do the following …
Change the details of an existing toxicity test	Change the appropriate cells in the worksheet 'Inventory of tests.'
Add a new toxicity test	1. Details of the new test must be updated in the list of tests in worksheet 'Inventory of tests'. If both screening and definitive tests exist, insert one row for each marking which is which, in the column headed 'Screening/Definitive'. 2. In worksheets 'RESOURCE TOX. TESTS', 'SOURCE TOX. TESTS FOR DISCHARGE', 'SOURCE TOX. TESTS FOR RESOURCE', 'Resource MM', 'Source MM Discharge' and 'Source MM Resource', copy and paste the whole of the last row to create two more rows.
Change management criteria	Change the appropriate cells in the relevant 'management criteria' worksheet.
Change matching rules	The user requirements (in either the RESOURCE CONTEXT or SOURCE CONTEXT) are reflected in row 6 in the worksheets 'RESOURCE TOX. TESTS', 'SOURCE TOX. TESTS FOR DISCHARGE' and 'SOURCE TOX. TESTS FOR RESOURCE'. Change these if necessary. The actual matching of the properties of each test with these requirements is done in the 'Resource MM', 'Source MM Discharge' and 'Source MM Resource' worksheets. The 'Included:' row marks those criteria that should be considered with a 1 (otherwise 0). To accommodate new matching rules, modify the formulae in the appropriate columns. (A test is recommended, *i.e.* marked with a 'Yes' in the 'RESOURCE TOX. TESTS', 'SOURCE TOX. TESTS FOR DISCHARGE' and 'SOURCE TOX. TESTS FOR RESOURCE' worksheets, if the overall count, in column B, equals the value appearing in cell A1).