USE OF INDIGENOUS RIVERINE INVERTEBRATES IN APPLIED TOXICOLOGY AND WATER RESOURCE-QUALITY MANAGEMENT

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Report to the Water Research Commission

by

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EXECUTIVE SUMMARY

Chapter 1: Introduction

1.1 Indigenous riverine invertebrates in toxicity testing

In South Africa, the stimulus for research into the tolerances of riverine organisms to water quality variables, was the recognition that 1) quantitative resource quality objectives could not be set for the rivers of the Kruger National Park with any degree of confidence; 2) the South African Water Quality Guidelines for Aquatic Ecosystems were entirely based on values from international toxicological databases; and 3) there were minimal experimental data for the responses of indigenous riverine invertebrates to water quality stressors.

Research on the tolerances of South African riverine invertebrates began, in support of the Kruger National Park Rivers Research Programme (KNPRRP), in 1992, when the Water Research Commission (WRC) funded the aquatic toxicology group of the Institute for Water Research (IWR) to develop an artificial stream laboratory. This was followed by investigations of the tolerances of South African riverine invertebrates to a range of variables, but with a focus on tolerances to inorganic salts (mainly sodium sulphate and sodium chloride) and complex wastewater. Attention was also paid to the selection and laboratory-breeding of South African invertebrates which could be used as toxicity test organisms. To date, there is no South African facility formally breeding indigenous invertebrates for routine toxicity tests, although the freshwater shrimp *Caridina nilotica* has proved promising. There is, however, the use of indigenous organisms for site-specific tests to derive site-specific guidelines or licence criteria.

Over the past decade progress has led to the Department of Water Affairs and Forestry (DWAF) preparing policy and technical documents, and pilot implementation using toxicity tests to set licence criteria for the discharge of complex wastewater discharges (Jooste, pers.comm.). It is envisaged that a tiered approach will be implemented, with tiers 1- 4 requiring the use of standard laboratory tests and organisms, and the final tier requiring a comprehensive ecological risk assessment which would include site-specific tests using riverine and other aquatic invertebrates. One of the most exciting developments has been the use of riverine organism toxicity test results in assessing environmental water quality requirements, as part of ecological Reserve determinations.

Internationally there is considerable interest in the tolerances of wild populations. Australia is the example most likely to be followed by South Africa. They have published a new generation of water quality guidelines for ecosystem protection; developed a database of indigenous tolerance-data; and there are frequent references to research on the tolerances of Australian invertebrates.

The main contributions made by the work presented in this report are:

1. The development of a South African database of the tolerances of riverine invertebrates
2. Contributions to the use of aquatic invertebrate toxicity test results in:
   - i) source directed controls (e.g complex wastewater discharge licensing), and
   - ii) resource directed measures (e.g. ecological Reserve assessments).

1.2 Legal and management context for the application of results

Introduction

The National Water Policy (DWAF, 1997), and the National Water Act (No 36 of 1998)(NWA) provide the legal and management context for the application of results. The law and the policy are founded on the concepts of equity (fairness of access to water and water services) and sustainability (the opportunity to optimally use water resources now and into the future) (NWA, 1(1)(xviii)(b)). The concept of sustainability is based on the understanding that on earth water comes packaged in aquatic ecosystems, and that the product, water, is intimately related to and affected by the structure and functioning of these ecosystems. (Aquatic ecosystems include rivers, lakes, wetlands, aquifers and estuaries. Impoundments act as artificial lakes connected to river systems.)

A key recognition during the development of the policy and the NWA was that "the environment" does not compete with users for resources - the environment (in this case aquatic ecosystems) ***is*** the resource. Therefore a key policy of DWAF is that of ***resource protection*** in order to achieve sustainable resource use. Resource protection is achieved through the implementation of resource directed measures (RDM) and source directed controls (SDC).

Resource Directed Measures (RDM)

RDM focus on the resource, and the qualitative and quantitative description of resource quality objectives (RQOs). Resource quality is defined in the NWA (1(1)(xix)), as including the quantity, pattern, timing and assurance of flow; the physical, chemical and biological characteristics of the water; the condition of instream and riparian habitat; and the condition and the distribution of aquatic biota. RQOs are qualitative and quantitative descriptions of the state that each of these components should achieve.

There are two important tools for implementing RDM: the South African Water Quality Guidelines for Aquatic Ecosystems; and the ecological Reserve. The Guidelines contribute towards defining the ecological Reserve which is defined as the quality and quantity of water required to protect aquatic ecosystems in order to secure ecologically sustainable development and use of aquatic resources (NWA 1(xviii)b).

The results of this project feed directly into the refinement and revision of aquatic ecosystem guidelines (a project which will be undertaken by DWAF within the next 5 years); and the current thrust to quantify the ecological Reserve. Much of the water quality method for ecological Reserve assessments has been based on WRC-funded research.

Source Directed Controls (SDC)

Source directed control focus on the control of impacts on the resource through the licensing of abstractions and discharges, and the setting of discharge standards. These licences and discharge standards will now be aimed at meeting specific instream resource quality objectives - resulting in a more integrated approach to environmental water quality management. The CAT-IWR database of indigenous invertebrate tolerances provides data for use in setting both licences and standards. DWAF will focus SDC implementation using toxicity testing in the first instance, on

complex wastewater discharges.

This project was co-funded by DWAF, and together with other WRC projects, has produced a range of interlinked products:

This project, "Use of riverine invertebrates in applied aquatic toxicology and water resource-quality management" was funded by the Institute for Water Quality Studies (IWQS, DWAF) and the Water Research Commission (WRC). Project funds from the WRC contributed to salinity tolerance testing for the Olifants River ecological Reserve assessment, and post graduate whole effluent toxicity (WET) testing studies of textile, pulp and paper, and complex industrial effluents. The IWQS, DWAF products were a standard protocol for single substance toxicity testing in flowing water, and an assessment of the method for WET testing in flowing water. Products from three other WRC projects have also been used in an integrated manner. SDC - Source Directed Control; CWD - complex wastewater discharge. CAT-IWR - Centre for Aquatic Toxicology, IWR

Funder	**Project**	**Product from project**	**Applications:**			
			Ecological Reserve methods	**Flowing water toxicity protocol**	**SDC of CWD**	**Instream toxicity monitoring**
WRC	Use of riverine invertebrates in applied toxicology	CAT-IWR database & variability	□			□
		Olifants River Reserve	□			
		Textile chronic WET testing			□	
	Sabie and Buffalo Rivers	Textile & Pulp/paper CWD acute WET testing			□	
	Vaal River WET tests	CWD acute WET testing			□	
	Water quality Reserve	Breede River Reserve	□			
IWQS - DWAF	Use of riverine invertebrates in applied toxicology	Methods for riverine test organisms: single substances	□			
		Methods for riverine test organisms: complex mixtures		□	□	
		SDC of complex wastewater discharges			□	

Environmental water quality management

South Africa will host the 2002 World Summit on sustainable development. Ecotoxicology is a valuable tool for sustainable environmental water quality management. In all developed and most developing countries the use of water resources for waste dilution and transport threatens other resource uses and users. In most developed countries toxicology and ecotoxicology are already harnessed as water resource management tools. In South Africa we have the opportunity to learn from the sophisticated, high-budget approaches of the US EPA, and to adapt these and other approaches to our circumstances.

South Africa has successfully implemented the national River Health Programme (RHP) (State of Rivers Report, 2001) which uses biotic indices to assess river health and to evaluate the achievement of biotic RQOs. Although biomonitoring results can alert water resource managers to a problem, they cannot be used to identify the cause of the problem. Water chemistry concentrations also do not in themselves provide information about ecosystem health. Ecotoxicology is the science which links the two. By assessing the responses of biota to chemical mixtures and single substance concentrations, ecotoxicity results have a key role to play in sustainable environmental water quality management.

This project provides methods for linking the results from RHP assessments, the national water chemistry database, and.ecotoxicity data, and also reports on case studies.

1.3 Links between this report and co-funded projects undertaken for the Department of Water Affairs and Forestry

This project has been conducted in close collaboration with the Institute for Water Quality Studies (DWAF) (see Table on previous page). Chapters 2 and 3, and Appendices 3 and 4 form products that are immediately required by DWAF. In addition, DWAF commissioned the publication of "A protocol for acute toxicity testing using selected riverine invertebrates in artificial stream systems" (Appendix 1.1). As part of the project we also contributed to the development of a discussion document and supporting technical document on the control of complex wastewater discharge impacts using a toxicity-based approach (Chapter 4).

1.4 Aims of the study

The general aims of the project were: to apply ecotoxicological research methods using indigenous riverine organisms as test taxa; to continue to develop methods of applying the results to the development and refinement of guidelines; and to further contribute to the development of water resource-quality policy, including the application of toxicological end-points in permit criteria.

Specific aims (location of reporting in bold):

1. Ecotoxicological research:

    a. To extend the number of variables and whole effluents which have been used for toxicity testing with indigenous riverine organisms (i.e. build up the database). This will be approached through the work of graduate students. (**Chapter 3,**

Appendices 3 & 4)

b. To apply the current toxicological database to setting general and site-specific guidelines for conductivity, with notes on specific ions, particularly sulphate.(**Chapter 2.2, Appendix 2**)

c. To synthesize and assess tolerance results, and to evaluate their usefulness in applied resource-quality management. (**Chapter 2**)

2. To contribute to the further development and application of the national water resource policy and specifically resource-quality policy. (**Chapter 4, Chapter 5**)

3 Collaboration and capacity building: (**Chapter 6**)

The project also aimed to link toxicology and the emerging development of ecological risk assessment (Claassen et al., 2001); and to participate in toxicological training. This included tertiary level teaching and running specific courses (**Chapter 6**).

As part of the collaboration with DWAF the authors produced "A protocol for acute toxicity testing using selected riverine invertebrates in artificial stream systems" (**DWAF, 2000; Appendix 1**).

Chapter 2: Review of salinity tolerance testing

2.1 The variability of responses from toxicity tests using indigenous riverine organisms

The aim of this section of Chapter 2 is to generate guidelines for incorporating and identifying variability of toxicity data using indigenous macroinvertebrates in artificial streams. Salinity toxicity data, with lethality as an end-point, where therefore interrogated and the variability of response data evaluated.

Many of the questions posed around variability remain unanswered, partly due to the levels of variability shown by the data, and the limitations of the small database of information available for this assessment. In many instances it was not possible to determine coefficients of variation (CV) and mean values had to be used. As expected, experiments using the standard laboratory organism *Daphnia pulex* show lower CV than when using indigenous invertebrates. The use of indigenous invertebrates as experimental organisms also has a greater effect on results than stream type. However, there does not appear to be a clear pattern relating mayfly data to the salt used for salinisation. When comparing area of origin of organisms, rivers generally show similar results, except for the Sabie River. The *Tricorythus* species in this river seems to be particularly sensitive, and would be a useful indicator for management if a protective approach was followed for this river.

It is recommended that the use of CVs for assessing the variability of CAT-IWR toxicity data - particularly for assessing experimental variability - be included in a Quality Assurance programme. The way in which variability testing is structured will depend on what data are being evaluated and the objective of the evaluation. The programme should include an evaluation with previous results, as well as internationally accepted variability parameters and data. It is understood that

acceptable variability margins may differ depending on the application of data, e.g. parameters of acceptability for use of data in site-specific Reserve assessments may be different to traditional concentration-response assessments, but acceptable variability values and data quality should be defined for each application. It is also recommended that as the WET testing database expands, variability methods specific to WET testing be evaluated, particularly as variability associated with an effluent is obviously higher than a single substance.

Due to the dependence of an accurate variability assessment on the volume and nature of available data, it is recommended that CAT-IWR staff also rely on other parameters for checking data validity, e.g. the application of appropriate acclimation and control mortalities, as well as the parameters listed below:

- χ^2 values - Probit model: These values are an assessment of how accurately the data being analysed fit the statistical model. US EPA version 1.5 produces both χ^2 tabulated and χ^2 calculated values. The general guideline is that if χ^2 calculated > χ^2 tabulated, then significant heterogeneity exists and data are not normally distributed. This result would not be valid.
- Confidence limits (CLs) - Probit and Spearman-Karber models: Upper and lower 95% confidence limits are produced, which are very useful in assessing data quality. The smaller the CLs around the LC50 or EC50, the more precise the data.
- % trim - Trimmed Spearman-Karber model: This indicates how much data has been trimmed off the extreme edges of the sigmoid concentration-response curve so as to best fit the model. The lower the % trim, the more accurate the data.
- Warning messages - Probit model: A number of warning messages are issued by Probit if the data being analysed do not fit the model accurately. These should be evaluated using professional judgment, as warning messages may not be issued even when the CLs are wide.

2.2 Application of salinity tolerance test results in ecological Reserve determinations

The project team has previously developed a hazard-based approach to applying site-specific salinity tolerance data in ecological Reserve assessments. This WRC project was used to research the development and application of the method in the Olifants River Ecological Water Requirements (OREWRA) study, and a WRC project on method development for the water quality aspects of ecological Reserve assessments allowed further development and testing in the Breede River ecological Reserve study.

Methods for the determination of water quality aspects of an ecological Reserve assessment were still under development at the start of the OREWRA study. The then current RDM method of relating water quality to resource health classes was successfully applied in the Crocodile River, but it proved to be unsatisfactory for the Sabie River. An alternative method was developed which allowed tolerance test data, rather than a percentage deviation from the reference condition, to link TDS values to resource classes. This was based on the understanding that elevated salinity acts as a slow toxicant, with both acute (96 hour) and chronic (10 day) responses recorded at concentrations well above those usually experienced in rivers.

The mayfly *Tricorythus discolor* is one of the dominant filter-feeding, riffle-dwelling macroinvertebrates in the Olifants River. *T. discolor* nymphs collected from a minimally salt-

impacted reference site were experimentally exposed to sodium sulphate since sodium sulphate is more toxic than sodium chloride, and sulphate was a dominant ion in many reaches of the Olifants River. From the results a hazard-based, site-specific salinity guideline for the upper reaches of the Olifants River, for resource classes A-F, was developed: The results of similar tolerance testing from the same genus of mayfly from the Sabie River were used to assess the Blyde River since it has naturally lower salinities, that are more similar to the Sabie River than to the main-stem of the Olifants River.

RELATIONSHIP BETWEEN RIVER CLASS AND SALINITY FOR ALL RIVER REACHES IN THE OLIFANTS STUDY AREA EXCEPT THE BLYDE RIVER		
Assessment class	**Electrical conductivity (mS/m)**	**Total dissolved solids (mg/l)**
A	20-35	130-195
B	35 - 45	195-293
C	45 - 80	293-520
D	80 - 120	520 - 780
E	120-155	780-1020
F	>155	>1020

RELATIONSHIP BETWEEN RIVER CLASS AND SALINITY FOR THE BLYDE RIVER BASED ON SABIE RIVER DATA		
Assessment class	Electrical conductivity (mS/m)	Total dissolved solids (mg/l)
A	8-17	50-100
B	17-30	100-195
C	30-40	195-260
D	40-50	260-325
E/F	>50	>325

The tolerance-based class relationship was a key step in the overall OREWRA water quality Reserve assessment, particularly in Steps 3 (Determination of reference conditions) and 4 (Assessment of the present ecological state (PES)).

The description of the OREWRA results is followed by a paper written for an international environmental flows conference, which describes the method used to link the tolerance test data, with biomonitoring results and chemical data:

> "Aquatic organisms have physico-chemical as well as flow habitat requirements. There are three main kinds of data which are useful in quantifying these

requirements: chemical data which describe instream physico-chemical conditions; ecotoxicity data which describe the tolerances of organisms and their instream responses to changing chemical concentrations; and biomonitoring data which describe the distribution and composition of communities. However, it is only by linking these data that organism requirements can be adequately elucidated and translated into resource quality objectives. This paper describes the methods for linking these data and their application in the integration of water quality into environmental flow assessments and the assessment of the water quality Reserve. A case study is presented to demonstrate the methodology." (Section 2.2.4) (Scherman et al., in prep.).

Chapter 3: Whole effluent toxicity (WET) testing

Acute WET tests provide a useful and important first screening step in assessing the toxicological effects of chemicals and effluents. It has been suggested that the systematic generation of toxicological data is a good approach to determine permissible limits of toxicants/effluents (Williams et al., 1984) and that site-specific application of these toxicity tests should be included (Chapman, 2000). Data presented in the studies confirmed that whole effluent toxicity tests using indigenous riverine invertebrates in artificial stream channels can be used to assess effluent discharge as organisms respond (increased mortality) to increased concentrations of effluent.

However, the presented studies also served to highlight issues and questions which contribute to variability in WET test and may result in confusion in the application of WET test results in water quality management. It should be stressed that these issues apply to toxicity tests in general and not necessarily only to the standard protocol described in this chapter; furthermore, issues raised are also pertinent to single substance and reference toxicant toxicity testing. It is only through further application of the standard protocol using both single substances and whole effluent that an attempt can be made to understand these issues.

Chapter 4: Contributions to policy development and implementation

There have been two main contributions to policy development and implementation.

1) The development of methods for assessing water quality within ecological Reserve assessments.

The task of an ecological Reserve assessment is to provide both quantified and descriptive information about the pattern and reliability of environmental flows, with information on flow frequency, magnitude and duration, so that an entire modified flow regime is provided. However methods for quantifying environmental water quality still focus on only magnitude (concentration), and frequency and duration are only taken into account via flow-concentration modelling. During an ecological Reserve assessment, the ecological Reserve for water quality is provided as class boundary-value concentrations for each variable.

This chapter describes the procedure required for assessing water quality in ecological Reserve determinations in a 5-step roadmap and discusses the process of integrating water quality with environmental flow assessments in Building Block Methodology (BBM) and Downstream Response to Imposed Flow Transformations (DRIFT) methods.

2) The development of a discussion document and supporting technical guidelines for the implementation of toxicity testing in the source directed control of complex effluent discharges.

This chapter describes a project which has made direct input into formalising the use of toxicity test results in pollution control by DWAF. The project team have worked in conjunction with DWAF researchers working on the development of guidelines for the assessment of complex wastewater discharges.

The management of pollution over the past four decades has largely been based on setting and managing levels of single substances in water. The success of this substance-specific approach in discharge management depends on knowledge of:

- the composition of the discharge, and
- knowledge of the effect of each component of the discharge.

In complex waste discharges either, or both of these, may be missing and therefore, in itself the control of single substances may not be effective in assessing the environmental hazard of discharges containing mixtures of substances.

The methodology described, i.e.Toxicity-based Ecological Hazard Assessment (TEHA), is an integrated and tiered approach to complex mixture assessment and is the equivalent of the USEPA whole effluent toxicity (WET) testing, the UK direct toxicity testing, and the Dutch complex effluent testing. The rationale of the TEHA approach as well as details of the tiered approach are discussed.

Chapter 5: Opportunities, recommendations and conclusions

5.1 Introduction

In 1994 Roux summarised the status of applied aquatic toxicology as follows:

> "To cope with increasing demands placed on the quality and quantity of aquatic resources, efficient management tools are continually required. Recent developments in environmental monitoring indicate the importance of incorporating biological indicators in assessment programmes. Aquatic toxicology has consequently become an important monitoring and regulatory science. Applications of aquatic toxicity testing include: deriving water quality criteria; toxicological evaluations of whole effluents and receiving waters; and the estimation of ecological risk. Toxicity testing can potentially play a significant role in improving water quality in years to come, especially through its application in effluent regulation. Currently however, few environmental laboratories in South Africa have the required expertise and facilities to carry out a representative range of toxicity tests. Training and funding are required to build the capacity for the necessary developmental research, before toxicity testing can routinely be implemented." Roux (1994).

It has proved to be difficult to realise this vision. Some progress is evident: Slabbert et al. (1998a; b) published appropriate methods; international ecotoxicity databases were used to derive aquatic ecosystem guidelines (DWAF,1996); and toxicology laboratories are more common (accredited

laboratories at the Institute for Water Quality Studies (DWAF) and Rand Water). However, in 2002 there is still no routine use of toxicity data in licensing and the application of source directed controls.

This project has however been part of key steps towards the realisation of this objective:

- In May 2000 the project team assisted the WRC in the organisation of a workshop for role-players where DWAF expressed a commitment to move towards implementation;
- in 2001 the aims and products of this project were shifted so that project team members could work with DWAF personnel in the production of a discussion document and a technical guideline for the use of toxicity testing in the management of complex wastewater discharges;
- in 2001 Ecological Risk Assessment guidelines were published (Claassen et al., 2001), citing the central role of toxicity testing in certain site-specific ERAs;
- in 2002 DWAF will release the discussion document and the technical guideline, and after Departmental discussions, a pilot implementation will be conducted; and
- in 2002 DWAF will initiate a national programme for instream toxicity monitoring.

Ecotoxicology can also contribute to resource directed measures:

- In 2002 DWAF will publish an ecological Reserve assessment manual which will recommend the inclusion of toxicity assessments within the water quality aspects of ecological Reserve assessments; and
- the national instream toxicity monitoring programme will also contribute to RDM.

This means there are real opportunities for using ecotoxicology in the implementation of both RDM and SDC. However, current initiatives are only the first steps. In both the RDM and SDC arenas there are areas of research that are critically important to successful implementation.

5.2 Recommendations

Some of the recommendations will be taken up as research objectives in the period 2002-2005. Where this is the case, the lead institution is listed in brackets at the end of the paragraph.

Environmental water quality and the use of toxicity testing in ecological Reserve assessments

General research requirements

The following areas of water quality within ecological Reserve assessment require specific research attention:

- The frequency and manner of water quality monitoring restricts the kinds of datasets that are available. At present, only concentration (magnitude) is considered, whereas frequency and duration also need to be taken into account. There is a need to see if water quality RQOs can be developed in a way which matches hydrological assessments in taking account of magnitude, duration and frequency of concentrations. To do this, the following actions are necessary: evaluation of current water quality databases for the development of acceptable time-series data for selected water quality variables; assessment of the applicability of a risk-based approach within the limitations of existing water quality data; and recommendation of optimal water quality monitoring in the light of emerging data requirements. Toxicity data can be used in time-series generation as they allow chemical

stressor-response relationships to be quantified (O'Keeffe et al., in press). (CAT-IWR).

- Ecological Reserve assessments rely heavily on expert opinion and judgement (King et al., 2000; in press) and in order to capture expert opinion and facilitate a systematic and consistent application of methods, an ecological Reserve Decision Support System (DSS) is being developed (Hughes, pers. comm). There is a need to integrate water quality data, tools, techniques and methodologies within this DSS. To do this, the following actions are necessary: Delphi code needs to be written that will allow the technical integration of existing water quality data, tools, techniques and methodologies within the DSS; water quality data formats need to be specified, and qualitative decision criteria need to be recorded. (CAT-IWR).

- Nutrient assessments require particular attention and nutrient criteria need to be selected (e.g. total phosphate, dissolved phosphate, total nitrogen or a ratio of two or more nutrients). The assumption in the Reserve that the reference condition in all ecoregions is equal to or less than the Target Water Quality Range in the South African Water Quality Guidelines for aquatic ecosystems (DWAF, 1996) needs to be validated and a better nutrient evaluation protocol needs to be developed. Nutrients are not toxic and it may be that zooplankton and invertebrate feeding behaviour or rates should be used as indices of health. (Freshwater Research Unit, University of Cape Town: FRU, UCT).

- The link between chemical, biotic, and toxicological data needs to be further developed and an assessment protocol written. The method for relating biomonitoring scores to ecological health classes needs to be formalised and recorded. (FRU, UCT).

- Methods for monitoring, assessing and relating turbidity and suspended solids levels to biotic responses and ecosystem health classes, need to be developed.

Salinity tolerance testing

South Africa is not alone in having salinity as a major water quality issue. In Australia the Government has pledged a large proportion of its research and water quality management funding to solving problems relating to salinisation (Nielsen and Hillman, 2000). South Africa has taken the international lead in the development of integrated methods for the quantifying of environmental flow requirements (King et al., 2000; in press).

The work presented in this report suggests that these two issues are linked. Dissolved ion concentrations and ratios in freshwater result in the first place from geology, and provide the basic chemical "fingerprint" of the water. Environmental water chemistry finally results from a combination of organic and inorganic processes. Different salts elicit different tolerance responses, and the tolerances differ regionally and between taxa.

Alternative methods for using salt tolerances in RDM, and methods for using salt tolerance data in SDC will depend on species- and site-specific data. It is important that the CAT-IWR salt tolerance database be used to erect hypotheses to 1) plan further tolerance experiments, and 2) erect hypotheses and test alternative approaches to modelling the relationship between salt tolerances and ecosystem health classes. (CAT-IWR).

Instream toxicity testing
The Olifants River study demonstrated that biomonitoring can reveal chemical stress not revealed by conventional chemical monitoring, and that this could then be linked to measurable instream toxicity (Palmer and Rossouw, 2001). It is important now to investigate sub-lethal effects, and to develop cost-effective methods for the evaluation of long-term sub-lethal effects. (CAT-IWR)

DWAF has already indicated a plan to implement instream toxicity monitoring. Ultimately the aim would be to link an understanding of invertebrate physiology, with the fate and effects of chemical mixtures in the environment and the ecology of freshwater systems.

Integration of environmental water quality and quantity requirements
It was obvious at the 2002 Environmental Flows for River Systems & Fourth International Ecohydraulics Symposium hosted by Southern Waters Ecological Research and Consulting, held in Cape Town in March 2002, that there has been little international attention paid to the integrated assessment and management of environmental water quality and quantity. It would be timeous for the South African water quality Reserve specialists to collaborate on a book which focusses on environmental water quality methods and applications. (CAT-IWR).

Environmental water quality and whole effluent toxicity (WET) testing

There is a strong likelihood that DWAF will implement complex industrial effluent management procedures using whole effluent (WET) toxicity testing, with a tiered approach called TEHA (Toxicity-based Ecological Hazard Assessment). For the envisaged Level 5 of TEHA, WET testing will need to be more sophisticated, with efficient, effective methods of assessing the sub-lethal, long-term ecological effects of complex mixtures. Ongoing work on sub-lethal measures, and the relationship between dilution and ecological health class will be essential. It will be important to develop methods and protocols to ensure that TEHA becomes part of routine ecological risk assessments of water quality impacts. (WRC Ecotoxicology Programme).

5.3 Conclusions

With regard to salt tolerances: riverine organisms are more tolerant of salt concentrations than are generally experienced under natural freshwater conditions. However, high salinities often have anthropogenic causes and can have lethal and/or sub-lethal effects. *Daphnia* are generally more sensitive to increased salt concentration than most freshwater invertebrates, but possibly not as sensitive as first instar or juvenile stages (e.g. freshwater shrimp neonates). The results of *Daphnia* tests are subject to the application of safety factors so as to ensure protection. It therefore makes economic sense to use *Daphnia* as the freshwater invertebrate in screening tests; riverine invertebrates for evaluations of intermittent, short term, high salinity events; and riverine invertebrates (using both juveniles or early instars and/or sub-lethal measures) for evaluating site-specific effects of sustained elevations on salinity.

In 1992 there was no information on the tolerances of South African riverine macroinvertebrates to either single substances or complex mixtures. There have been considerable developments in ecotoxicology since then: CAT-IWR has a South African riverine invertebrate tolerance database; the WRC has an Ecotoxicology research programme; and DWAF has plans for the active use of ecotoxicology in both RDM and SDC. This project has contributed both to the development of RDM and SDC water quality assessment methods, and to the management and policy frameworks

which direct the use of ecotoxicology.

Although further method development is required, there is also a need for a period of critical review and consolidation, particularly with regard to the generation, curation, and publication of tolerance data of South African invertebrate taxa. The science of ecotoxicology is currently well-placed to contribute to environmental quality management both in South Africa and internationally.

Chapter 6: Collaboration and capacity building

6.1 Research collaboration and contributions

The project team have collaborated with a wide range of people and institutions, but there are researchers who have more than collaborated - they have significantly contributed to this report. They are: Dr Sebastian Jooste, DWAF , IWQS: critical discussion of all aspects of the project, development of an alternative use of salinity tolerance data in ecological Reserve assessments, and co-writing of the technical guideline for TEHA; Dr Heather Malan FRU,UCT: critical discussion of all aspects of the project, and development of a model which related flow to instream salt concentrations; Mr Nico Rossouw, Ninham Shand critical discussion of all aspects of the project, and co-development of all the water quality Reserve methods; and Dr Dirk Roux, CSIR: evaluation of the project products and the development of an objectives hierarchy and adaptive management plan for future research.

Our graduate students have stimulated our thinking and contributed data and reports:
Mrs Tandiwe Zokufa worked on the acute effects of textile and pulp and paper effluents (Zokufa et al., 2001), Appendix 3, and Ms Vicky Everitt worked on complex industrial effluents (Everitt, 1999). Mrs Heather Davies-Coleman began her doctoral work on the reproduction of a freshwater limpet which allowed her to evaluate the sub-lethal effects of textile effluent. Honours students Mr Thabani Mlilo, Ms Nolwazi Mkize, Mr Lindela Tshwete Ms Sandy Dlamini and Ms Sekiwe Mbande all did projects which contributed to this research.

Institutions with which we have collaborated include: Departments within Rhodes University; DWAF; University of Cape Town - Freshwater Research Unit; University of Transkei; Unilever UK; Lever Ponds South Africa; Richards Bay Minerals; SASOL; CSIR;

6.2 Co-funding

The two main funders were the WRC and DWAF-IWQS. The products of a range of associated projects are listed in the Table on Page iii.

6.3 Capacity building

Employment and development of staff from designated groups
Mr Phehello Mahasele joined CAT-IWR in March 2000. He has a BSc Honours degree in Leather Science and completed the CAT-IWR certificated course in Introductory Applied Aquatic Toxicology in May 2000. He was trained in laboratory methods required for the use of *Daphnia* spp. and indigenous riverine organisms in aquatic toxicity testing; participated in consulting projects; and was registered for an MSc on sub-lethal testing of complex wastewater discharge.

Mr Mahasele suffered a tragic motor accident in July 2001, and was unable to work from then on. His MSc studies have been suspended.

Ms Michele Stewart was a research assistant in 2000 and 2001. Ms Stewart is a mature student, studying part-time, and as a severe epileptic can be considered disabled.

Funding and supervision of post-graduate students from designated groups.
The following BSc Honours students have all completed courses and/or projects in applied aquatic toxicology: Mr Thabani Mlilo, Ms Nolwazi Mkize, Ms Sekiwe Mbande, Ms Nomahlube Sishuba, Ms Kim Wilkins, Ms Sandy Dlamini and Mr Lindela Tshwete.

Co-operative research ventures with previously disadvantaged institutions (PDIs).
Mr Bonani Madikizela, previously from UNITRA (but now with IWQS, DWAF) is undertaking tolerance testing of riverine invertebrates to elevated turbidity as part of his doctorate, which is being co-supervised by Dr Scherman.

Partnering staff from government departments
The project team has worked with the Institute for Water Quality Studies (IWQS, DWAF) to pilot a partnership programme where we have effected direct knowledge and technology transfer by interacting with selected IWQS staff. Mrs Tandiwe Zokufa and Mr Malesela Papo have both worked on ecological Reserve assessments with staff of CAT-IWR.

Community involvement
The project team was involved in an investigation of the effects of in-stream detergent use. Mr Malixole Soviti completed an MSc in January 2002 on community-use of detergents for in-stream washing. This work builds on WRC-funded community research conducted by Ms Nicole Motteux (Geography Department, Rhodes University)

Capacity-building of research personnel, industry and managing agencies from designated, as well as non-designated groups.
CAT-IWR has a well-developed network of interactions with other researchers and practitioners in aquatic toxicology, and participate in the Aquatox Forum. We initiated a WRC-funded national workshop on applied aquatic toxicology and risk assessment, and are involved in ongoing communication about toxicology with other researchers, DWAF and industry. We have developed course-ware in applied aquatic toxicology and in the 2 courses that have been offered, more than 50 % of the delegates were from designated groups.

Contracts
CAT-IWR is regularly involved in consulting work in the field of applied aquatic toxicology and environmental water quality. We are committed to using these opportunities for capacity building of our staff, students and associated colleagues. To date Mr Phehello Mahasele, Mrs Tandiwe Zokufa, Mr Malesela Papo, Mr Tobile Bokwe, and Mr Bonani Madikizela have worked with us on CAT-IWR contracts in a capacity building relationship.

Lecturing
The project team contributes substantially to the Applied Freshwater Ecology semester course. The emphasis of the course is on natural resource protection and sustainable use and uses as its framework the environmental protection policy of the 1998 National Water Act. It is offered at

third year level to students in the Departments of Zoology and Entomology, Environmental Science, Chemistry, Microbiology, Geography, Ichthyology and Fisheries Science and Botany. Prof Palmer also lectures part of a Water Resource Management module, i.e. an honours level course taught in the Department of Geography. The project team lectures a module on Applied Aquatic Toxicology to honours students registered in the Departments of Zoology and Entomology, Environmental Science and Geography. The project team also teaches an intensive week of Applied Aquatic Toxicology to students registered for an MSc in Biotechnology at Rhodes University (Department of Biochemistry and Microbiology).

Masters and doctoral graduates and students

Mrs H Davies-Coleman, **PhD, Graduated 2002**: The growth and reproduction of the freshwater limpet *Burnupia stenochorias* (Pulmonata, Ancylidae), and an evaluation of its use as an ecotoxicological indicator in whole effluent testing.

Mr M Claassen: **PhD**: The development of an Ecological Risk Assessment methodology for application in South African water resource management

Mr B Madikizela **PhD**: The effects of suspended sediment on macroinvertebrates in the Umzimvubu River, Eastern Cape, South Africa.

Mr M Binder **MSc, Graduated 1999**: An evaluation of recirculating stream designs for acute toxicity testing using two South African Ephemeroptera species exposed to sodium sulphate.

Ms V Everitt **MSc, Graduated 1999**: The use of indigenous macroinvertebrates and *Daphnia pulex* in acute toxicity testing.

Mr MK Soviti **MSc, Graduated 2002**: Investigating the impact of the in-stream use of detergents on water quality in the upper Kat River valley, Eastern Cape.

Ms Z Mabaso **MSc**: The project is in its early stages, but the biological and physical indices developed to monitor the two rivers are expected to be widely applicable within the Eastern Cape.

Mr P Mahasele *Degree suspended July 2001 due to motor accident.* Sub-lethal and chronic toxicity responses of the freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae) to sodium sulphate

Short Courses

The *Unilever Course in Applied Aquatic Toxicology* is the major CAT-IWR capacity building initiative, to which all staff contribute. It is an intensive one-week course, aimed at mid-level managers and staff from regulatory agencies, consultancies and industry, and includes a balance of lectures and practicals in order to provide a comprehensive overview of the use of aquatic toxicology in water resource management. The course in 2001 was co-ordinated by Dr Nikite Muller, and took place in May. Participants included 11 Rhodes MSc Biotechnology students (for whom the course is a compulsory module); 4 CAT-IWR honours students (for whom the course is followed by four intensive 3-hour tutorials); and 6 professionals (mainly from the regulator - DWAF, and industry). Half the delegates were female, and half from previously disadvantaged groups.

The *National Short Course on the role and use of Aquatic Biomonitoring* has been hosted by the IWR since 1999 and is co-ordinated by Drs Patsy Scherman and Nikite Muller. The aim of the course is to provide an understanding of the concepts of different biomonitoring techniques and

demonstrate how they can be used in water resource management. The course requires the coordination of more than 20 lecturing staff, including members of CAT- IWR, and management of a timetable which includes lectures and both field and laboratory practicals. The course is attended mostly by staff from regulatory agencies, consultancies and industry, although students from various tertiary institutions have attended. In 2001 there were 35 delegates.

Publications, reports and presentations produced by project team members July 1998 - June 2001 (listed in full in Chapter 6)

These include: 4 full-length papers in specialized technical journals and books ; 20 reports; and 20 conference presentations and posters.

Appendix 1: Acute toxicity test protocol and the CAT-IWR database
The acute toxicity test protocol was published in 2000 by DWAF and describes acute toxicity tests using riffle-dwelling invertebrates as test organisms in recirculating artificial streams. The protocol provides specific formalised instructions and a standard method for the use of riverine invertebrates as aquatic toxicity test organisms.

A description of the toxicity database, developed at CAT-IWR, is provided. The database was developed in Microsoft Access and is therefore linked to the Microsoft Suite of programmes (i.e. Word and Excel) in order to allow for data analysis and reporting.

Appendix 2: Salinity tolerance data
This appendix includes a table of data extracted from the CAT-IWR database to assess data variability for Chapter 2.1.

Appendix 3: WET - Kraft effluent and textile study
The aim of the study was to investigate the acute tolerances of selected riverine invertebrates to complex saline effluents from kraft (pulp and paper) and textile processing. Presently, there is very little information on indigenous organism response to saline effluents.

Following the standard protocol (DWAF, 2000) a series of WET tests were undertaken. WET tests can also be used by industry to quantify the responses of riverine biota to particular chemical constituents and complex whole effluents. Both *T.tinctus* and baetid test organisms proved to be suitable test organisms for toxicity testing. However, they should be considered as test organisms for auditing purposes, as their availability cannot always be guaranteed. Regular toxicity testing using a standard organism such as *Daphnia* should be included in effluent management programmes. Indigenous riverine invertebrates should be used to set guidelines for ecosystems, as they are representative of the impacted aquatic environment, thereby allowing a more direct prediction of effects.

To more effectively protect the environment, the management structures of both kraft and textile mills should consider adopting Ecological Risk Assessment programmes, as their effluents are toxic and warrant more appropriate management. The management would have to be conservative in the manner in which they treat or dispose of their effluent, and should target zero impact. Both effluents should be treated before irrigation to reduce variables that contribute to toxicity. As salinity has been identified as a problem in the Buffalo River, the discharge of saline effluents in aquatic environment should be prohibited.

Appendix 4: WET - Vaal River Study

The project aimed to investigate and compare the responses of a standard test organism and site-specific indigenous invertebrates to a range of chemical mixtures in order to evaluate the use of WET testing in water quality management. Specific aims of the project were to use *Daphnia pulex* and selected indigenous invertebrates, from the Vaal River area, as test organisms and to relate the toxicity test results to the application of WET testing in water quality management.

Standard protocols for toxicity tests using *D. pulex* and indigenous invertebrates (two species of mayfly) were followed. Results showed that species differed considerably in their sensitivity to toxicants and that the use of site-specific indigenous invertebrates in toxicity tests allows for a more accurate prediction of the effects of pollutants in the receiving water (Vaal River).

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GLOSSARY

The definitions listed here have been compiled from a variety of sources, including Rand and Petrocelli, 1985; DWAF, 1996; Lamberti and Steinman, 1993; Rand et al., 1995; Palmer et al., 1996; Binder, 1999; US EPA, 2000.

acclimation — Time period prior to the initiation of a toxicity test in which aquatic organisms are maintained in untreated, toxicant-free dilution water with physical and chemical characteristics similar to those to be used during the toxicity test.

acute tests — Short-term toxicity tests of 4 days or less; mortality is the response measured.

artificial stream — Any constructed channel that has a controlled flow of water and is used to study a physical, chemical or biological property of natural streams.

benchmark — A recognisable end-point, or condition that can be commonly identified, for example the natural condition, a threshold of probable concern or the chronic effects value.

bioavailability — The extent to which a particular constituent is available to the biota.

chemical species — The various chemical forms (e.g. ionic forms) of an element.

chronic tests — Involves a stimulus that is lingering or continues for a long period i.e. from several weeks or months to years, depending on the life cycle of the organism.

coefficient of variation (CV) — A standard statistical measure of the relative variation of a distribution or set of data, defined as the standard deviation divided by the mean (also called the relative standard deviation or RSD).

complex waste discharge — A discharge of wastewater of which the composition and/or impact cannot be assessed.

concentration-response curve — A curve describing the relationship between different exposure concentrations of a material and percentage response of the exposed test population.

dilution water (diluent) — Water used to dilute or prepare the test material; the control is dilution water only.

DWAF — Department of Water Affairs and Forestry.

EC — Electrical conductivity i.e. the measure of electrical current conducted which depends on the ions in solution, and is also therefore a measure

	of the total quantity of salts dissolved in a sample of water
EC50	Effective concentration at which 50% of the test organisms experience an adverse effect
end-point	The adverse biological response that is measured, and used as criteria for effects.
hazard	Having the potential to cause (often a specific) undesirable effect.
KNP	Kruger National Park
LASU	Large Artificial Stream Units
LC50	Concentration that kills 50% of the population observed. Also known as median lethal concentration (dose).
logistic regression	A type of statistical regression model that estimates the probability of an event occurring using one or more predictor variables, and is often used in concentration-response relationships.
macro-invertebrates	Invertebrates that are retained by mesh sizes ≥200 to 500μm.
mayflies	A group of insects with aquatic nymphs, generally sensitive to polluted conditions e.g. Order Ephemeroptera, families Tricorythidae, Baetidae and Leptophlebiidae.
Minimum significant difference (MSD)	The magnitude of difference from the control where the null hypothesis is rejected in a statistical test comparing a treatment with a control. MSD is based on the number of replicates, control performance, and power of the test.
multispecies toxicity test	Any test at a level of biological organization higher than a single species.
NOEC	No observed effect concentration.
nymph	The immature stage (no functional wings) of insects that do not have a pupal stage in the life-cycle (e.g. mayflies).
parameter	One of a set of measurable factors that define a system and determine its behaviour.
probit analysis	A statistical procedure used to analyse data arising from toxicity tests. The response measured is transformed into 'probit' values.

Resourse quality

The quality of all the aspects of a water resource including:

a.) the quantity, pattern, timing, water level and assurance of instream flow;

b.) the water quality, including the physical, chemical and biological characteristics of the water;

c.) the character and condition of the instream and riparian habitat; and

d.) the characteristics, condition and distribution of the aquatic biota.

risk

The likelihood of experiencing a specific undesirable effect.

salinisation

The process by which the saltiness of soils and rivers increase.

Spearman-Karber (S-K)

A statistical procedure used to analyse data arising from toxicity tests.

standard error

A measure of the precision of an estimate.

static system

An exposure system in which the test chambers contain still solutions of the test material or control water.

statistically significant effects

Responses in the test population that are different from those in the controls at a given statistical probability level, usually $P \leq 0.05$.

sub-chronic test

Four to 7-day toxicity test.

sub-lethal

Below the concentration that directly causes death; producing less obvious effects on behaviour, biochemical and/or physiological function.

TEHA

Toxicity-based ecological hazard assessment (TEHA) is an integrated approach to complex waste discharge assessment.

toxicant

An agent or material capable of producing an adverse response (effect) in a biological system, seriously injuring structure and/or function or producing death.

toxicity

The inherent potential or capacity to cause adverse effects in a living organism upon exposure.

toxicity curve

The curve obtained by either plotting the median survival times of a population of test organisms against test material concentrations or median effective concentrations against exposure times. Toxicity curves must provide an indication of the threshold median concentration. Unless toxicity curves are obtained and lethal thresholds

established, the results of any toxicity test must be treated with caution.

water quality guideline — A scientifically-based set of prescriptions to provide a management framework for implementing water quality criteria, including the criteria, background information, information on the fate and effects of the substance, specifications for monitoring and analyses etc.

water quality criteria for aquatic ecosystems — Numerical values or narrative statements that are calculated from experimental data and based on expert opinion, with the aim of protecting the aquatic environment.

ACKNOWLEDGEMENTS

Sincere thanks to all the contributors and collaborators, and to the steering committee, several of whom were actively involved in the project: Dr S Jooste, Dr H Malan, Mr N Rossouw, Dr H du Preez, Dr D Roux, Mr M Claassen, Prof J van Vuren, Dr L Phillips, Mr M Graham, Dr C Dickens.

Special thanks to Dr Steve Mitchell and to Drs Anneli Kühn and Sebastian Joose (IWQS, DWAF) for sharing our vision of research in support of actively applied aquatic toxicology. We look forward to our future collaborations. Mr Bill Rowlston (Strategic Planning, DWAF) provided critical discussions, particularly of implementation options.

The Kruger National Park, and particularly Dr Freek Venter and Mr Jacques Venter helped with the collection of mayfly larvae from the Olifants River. The personnel at the Worcester DWAF office kindly provided space and logistical support for the running of toxicity experiments for the Breede River study.

All members of the Institute for Water Research were enthusiastic and helpful colleagues. The support staff contributed significantly: Mrs Juanita McLean, Mr David Forsyth, Dr Gay Youthed, Ms Kholiswa Ralo and Mrs Lynn Marajh.

All the industries with whom we collaborated were actively helpful, and unencumbered funding from Lever Ponds was used substantially in support of this project.

The last weeks of this project were spent preparing for the 2001 conference of the Southern African Society of Aquatic Scientists. Our Junior Research Officer and Masters student, Phehello Anthony Mahasele, suffered a serious motor accident on his way to present his first academic poster. He has had to suspend his studies and research activities since then. Phehello, from all the rest of team, thank you. From our work with you on this project, we will always remember your enthusiasm, helpfulness and your wide smile. We deeply admire your courage and determination following the accident and thank you for the contribution you made to this work.

Finally, as always, thanks to friends and family.

CHAPTER 1

INTRODUCTION

1.1 INDIGENOUS RIVERINE INVERTEBRATES IN TOXICITY TESTING

In South Africa, the stimulus for research into the tolerances of riverine organisms to water quality variables was the recognition that quantitative resource quality objectives could not be set for the rivers of the Kruger National Park with any degree of confidence (Moore, 1990). The South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996) were entirely based on values from international toxicological databases. There were no experimental data for the responses of indigenous riverine invertebrates to water quality stressors.

The Kruger National Park Rivers Research Programme (KNPRRP) (Breen et al., 2000) provided the focus of freshwater research in the late 1980's and the 1990's. Research on the tolerances of riverine invertebrates began, in support of the KNPRRP, in 1992 when the Water Research Commission (WRC) funded the aquatic toxicology group of the Institute for Water Research (IWR) to develop an artificial stream laboratory (Palmer et al., 1994). This was followed by investigations of the tolerances of South African riverine invertebrates to a range of variables, but with a focus on tolerances to inorganic salts (mainly sodium sulphate and sodium chloride) and complex wastewater discharges (Palmer et al., 1996; Williams, 1996; Palmer and Goetsch, 1997; Goetsch and Palmer, 1997; Gerhardt and Palmer, 1998; Binder, 1999; Everitt, 1999; Palmer and Scherman, 2000; Zokufa, 2001; Davies-Coleman, 2001). Also at the IWR, Haigh and Davies-Coleman (1997,1999) paid attention to the selection and laboratory-breeding of South African invertebrates which could be used as toxicity test organisms; while method development and the application of toxicology using more conventional tests and test-organisms also received attention from the CSIR (Slabbert et al., 1998a; b).

To date, there is no South African facility breeding indigenous invertebrates for routine toxicity tests, but there is the use of wild-caught organisms for site-specific tests, to derive site-specific guidelines or licence criteria (Palmer, 2002). The Department of Water Affairs and Forestry (DWAF) are preparing policy and technical documents, and pilot implementation using toxicity tests to set licence criteria for the discharge of complex wastewater discharges. It is envisaged that a tiered approach will be implemented, with tiers 1- 4 requiring the use of standard laboratory tests and organisms, and the final tier requiring a comprehensive ecological risk assessment (Claassen et al., 2001) which would include site specific tests using indigenous invertebrates (Palmer and Scherman, 2000). One of the most exciting developments has been the use of indigenous toxicity test results in assessing environmental water quality requirements as part of ecological Reserve determinations (Palmer and Scherman, 2000; Palmer et al., 2000; Palmer and Rossouw, 2001).

Internationally there is considerable interest in the tolerances of wild populations. Mesocosms are frequently used to assess the responses of invertebrate communities (for example: Shaw and Manning, 1996; Gruessner and Watzin, 1996; Dieter et al., 1996; Gillespie et al., 1996; Dorn et al., 1997; Dyer and Belanger, 1999; Richardson and Kiffney, 2000; Schluz and Liess, 2001a). Aquatic insects are less frequently specifically investigated (Hickey and Clements, 1998; Pastershank et al., 1999). Attention had been paid to sub-lethal effects (for example: van der Geest et al., 1999; Sherratt et al., 1999; Balch et al., 2000; Schulz and Liess, 2001b), and bioaccumulation (Bervoets et al., 1996; Rousch et al., 1997; Hickey and Clements, 1998). Australia is the example most likely to be followed by South Africa. They have published a new generation of water quality guidelines for ecosystem protection (Hart et al., 1999; ANZECC and ARMCANZ, 2000), developed a database of indigenous tolerance data (Warne et al., 1998,

1999) and there are frequent references to research on the tolerances of Australian invertebrates (for example: Smith et al., 1999; Hodge et al., 2000; Boonthai, 2000).

Thus, the main contributions made by the work presented in this report are:

1. The development of a South African database of the tolerances of riverine invertebrates
2. Contributions to the use of toxicity test results in
   i) source directed controls (e.g complex wastewater discharge licensing) and
   ii) resource directed measures (e.g. ecological Reserve assessments).

1.2 LEGAL AND MANAGEMENT CONTEXT FOR THE APPLICATION OF RESULTS

1.2.1 Introduction

The National Water Policy (DWAF, 1997), and the National Water Act (No 36 of 1998) provide the legal and management context for the application of results. The law and the policy are founded on the concepts of equity (fairness of access to water and water services) and sustainability (the opportunity to optimally use water resources now and into the future) (NWA, 1(1)(xviii)(b)). The concept of sustainability is based on the understanding that on earth water comes packaged in aquatic ecosystems, and that the product, water, is intimately related to and affected by the structure and functioning of these ecosystems. (Aquatic ecosystems include rivers, lakes, wetlands, aquifers and estuaries. Impoundments act as artificial lakes, connected to river systems.)

A key recognition during the development of the policy and the NWA was that "the environment" does not compete with users for resources - the environment (in this case aquatic ecosystems) ***is*** the resource. Therefore a key policy of DWAF is that of ***resource protection*** in order to achieve sustainable resource use. Resource protection is achieved through the implementation of resource directed measures (RDM) and source directed controls (SDC).

1.2.2 Resource Directed Measures (RDM)

RDM focus on the resource, and the qualitative and quantitative description of resource quality objectives (RQOs). Resource quality is defined in the NWA (1(1)(xix)), as including the quantity, pattern, timing and assurance of flow; the physical, chemical and biological characteristics of the water; the condition of instream and riparian habitat; and the condition and distribution of aquatic biota. RQO's are qualitative and quantitative descriptions of the state that each of these components should achieve.

There are two main implementing actions for RDM : the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996); and the ecological Reserve (DWAF, 1999 - and under current revision). The Guidelines are part of, and contribute towards defining the ecological Reserve. The ecological Reserve is defined as the quality and quantity of water required to protect aquatic ecosystems in order to secure ecologically sustainable development and use of aquatic resources (NWA 1(xviii)b).

The results of this project feed directly into the refinement and revision of aquatic ecosystem guidelines (a project which will be undertaken by DWAF within the next 5 years); and the current thrust to quantify the ecological Reserve. Much of the water quality method for ecological

Reserve assessments (Palmer et al., 2000; DWAF, in prep - revision of DWAF, 1999) has been based on WRC-funded research (Palmer and Scherman, 2000; Malan and Day, in prep.; this report).

1.2.3 Source Directed Controls (SDC)

Source directed control focuses on the control of impacts on the resource through the licensing of abstractions and discharges, and the setting of discharge standards. These licences and discharge standards will now be aimed at meeting specific instream resource quality objectives - resulting in a more integrated approach to environmental water quality management. The CAT-IWR database of indigenous invertebrate tolerances (Appendix 1.2) provides data for use in both licences and standards. DWAF will focus SDC implementation using toxicity testing in the first instance, on complex wastewater discharges (DWAF, in prep.).

This project was co-funded by DWAF (see Chapter 6) and one of the main products for DWAF was the production of a technical guideline, and a discussion document on the use of toxicology in SDC, specifically the licensing of complex wastewater discharges.

1.2.4 Environmental water quality management

South Africa will host the 2002 World Summit on sustainable development. Ecotoxicology is an important tool for sustainable environmental water quality management. In all developed and most developing countries the use of water resources for waste dilution and transport threatens other resource uses and users. In most developed countries toxicology and ecotoxicology are already harnessed as water resource management tools. In South Africa we have the opportunity to learn from the sophisticated, high-budget approaches of the USEPA, and to adapt these and other approaches to our circumstances. South Africa has successfully implemented the national River Health Programme (RHP) (State of Rivers Report, 2001) which uses biotic indices to assess river health and to evaluate the achievement of biotic RQO's. Although biomonitoring results can alert water resource managers to a problem - they cannot be used to identify the cause of the problem. Water chemistry concentrations also do not in themselves provide information about ecosystem health. Ecotoxicology is the science which links the two. By assessing the responses of biota to chemical mixtures and single substance concentrations, ecotoxicity results have a key role to play in sustainable environmental water quality management.

This project provides methods for linking the results from RHP assessments, the national water chemistry database, and ecotoxicity data, and also reports on case studies.

1.3 Links between this report and co-funded projects undertaken for the Department of Water Affairs and Forestry

This project has been conducted in close collaboration with the Institute for Water Quality studies (DWAF) (see Chapter 6). Chapters 2 and 3, and Appendices 3, 4 and 5 form products that are immediately required by DWAF.

In addition, DWAF commissioned the publication of "A protocol for acute toxicity testing using selected riverine invertebrates in artificial stream systems" (DWAF, 2000; Appendix 1) which was written by Scherman and Palmer.

As part of the project we also contributed to the development of a discussion document and a supporting technical document on the control of complex wastewater discharge impacts using a toxicity-based approach (Chapter 4; DWAF, in prep.).

1.4 AIMS OF THE STUDY

The general aims of project were: to apply ecotoxicological research methods using indigenous riverine organisms as test taxa; to continue to develop methods of applying the results to the development and refinement of guidelines; and to further contribute to the development of water resource-quality policy, including the application of toxicological end-points in permit criteria.

Specific aims, and location of reporting in bold:

1. Ecotoxicological research:

    a. To extend the number of variables and whole effluents which have been used for toxicity testing with indigenous riverine organisms (i.e. build up the database). This will be approached through the work of graduate students. (**Chapter 3, Appendices 3 & 4**)

    b. To apply the current toxicological database to setting general and site-specific guidelines for conductivity, with notes on specific ions, particularly sulphate. (**Chapter 2.2; Appendix 2;** Palmer, 2002; Palmer and Rossouw, 2001)

    c. To synthesize and assess tolerance results, and to evaluate their usefulness in applied resource-quality management. (**Chapter 2, Appendix 5**)

2. To contribute to the further development and application of the national water resource policy and specifically resource-quality policy. (**Chapter 4, Chapter 5**)

3 Collaboration and capacity building. (**Chapter 6**)

The project also aimed to link toxicology and the emerging development of ecological risk assessment (Claassen et al., 2001); and to participate in toxicological training. This included tertiary level teaching and running specific courses. (**Chapter 6**)

As part of the collaboration with DWAF the authors produced "A protocol for acute toxicity testing using selected riverine invertebrates in artificial stream systems". (**DWAF 2000; Appendix 1.1**)

CHAPTER 2

REVIEW OF SALINITY TOLERANCE TESTING

2.1 THE VARIABILITY OF RESPONSES FROM TOXICITY TESTS USING INDIGENOUS RIVERINE ORGANISMS

2.1.1 Introduction

An assessment of variability of responses during toxicity testing is necessary if toxicological methods are to be used in the regulatory management of water resources. The Department of Water Affairs and Forestry (DWAF) is currently developing a technical document outlining the use of toxicological methods for dealing with complex waste streams. In addition to the development of a protocol of methods (completed in 2000), it is necessary to evaluate the variability of responses and the acceptability of toxicity data.

Variability is inherent to biological organisms, particularly if indigenous field-collected organisms are used for toxicity testing. This variability may be reduced by the meticulous selection and collection of experimental organisms for testing, and the precision of staff conducting the toxicity tests (DWAF, 2000 - Section 2.9: Quality assurance). When assessing the variability of responses of macroinvertebrates during toxicity testing, variability related to the following natural and experimental parameters must be assessed, and the contribution of each factor to variability determined:

- test organism
- toxicant (the nature of the salt used to simulate increasing salinisation)
- diluent (the experimental medium in which the experiment was conducted e.g. dechlorinated tap water vs. river water)
- area and river of origin of the test organism
- stream type and experimental design

Test organism

Variability can be ascribed to factors such as genetic variation, age, stage of life-cycle, sex, general health and physiological fluctuations inherent to the organism. Organisms are also affected by changes in their biotic and physical environments. Variability in the responses of test organisms is therefore due to the nature and state of the organism itself, as well as variability linked to the use of test organisms as a sample of the broader population (Rothery, 2000). The latter is particularly valid when using results from toxicity tests of single or few test species, to represent the tolerances of a suite of organisms, e.g. in ecological Reserve assessments (Section 2.2). This application can only be valid if the sensitivities of the tested organisms are within the same range as the other affected organisms of the same group (e.g. macroinvertebrates) at that site. Safety factors, or more protective results e.g. a LC1 rather than a LC5 value, can be applied to toxicity data in an attempt to compensate for single species tests. Care must be taken that results are not then over-protective, particularly as effects in the laboratory are often more severe than those seen in the field (Rand, 1995).

Toxicant

Research at CAT-IWR has focussed on the use of NaCl to represent increasing *salinisation* from agricultural run-off, and Na_2SO_4 to represent mining effluent (Palmer and Scherman, 2000). When salinity testing is conducted, the salt selected as toxicant is based on the nature of land-

uses in the catchment from which the organisms are sourced and therefore the probable cause of increasing salinisation, and the purpose of the experiment. Results of these experiments (e.g. LC50 values) are expressed as electrical conductivity (EC), total dissolved solids (TDS), and the salt in question (e.g. NaCl). Mixtures of salts have also been used for specific purposes; and experiments using $MgSO_4$ and $CaSO_4$ as toxicant. As the purpose of this research is to determine macroinvertebrate tolerances to salinisation, it is important to understand the significance of using different salts to simulate salinisation. Is it appropriate to generate general water resource guidelines for managing salinity, or must the guidelines be salt-specific? This question can only be answered by interrogating toxicity data conducted over time, using a number of different organisms, and a number of different salts, and evaluating the variability of toxicity data.

Diluent

Previous studies using *Daphnia pulex* have shown differences in response depending on the dilution water used for toxicity testing (Everitt, 1999; Scherman and Muller, 2001; Muller and Palmer, 2002). It is necessary to check the significance of this effect, as experiments using macroinvertebrates are not run in more than one diluent, due to practical difficulties. The use of site-specific river water as diluent incorporates the variability associated with changing river conditions, necessitating chemical speciation studies for many toxicants. Speciation of salts in Sabie River water (i.e. relatively unimpacted river water with a simple chemical profile) using the speciation programme JESS (Joint Expert Speciation System) did not show a major effect on toxicity; recorded mortalities were due to increasing osmotic pressure, related to increasing EC and ionic strength (Scherman et al., in press). The particular salt used to increase salinity may have additional effects, e.g. Na has a synergistic effect and Ca an antagonistic effect (Scherman et al., in press), but mortality is largely due to increasing osmotic pressure, which is related to increasing EC and ionic strength. For this study, it is therefore necessary to verify whether the variability in response related to the use of diluent is significant, or whether it falls within the range of natural variability associated with the use of macroinvertebrates as experimental organisms for toxicity testing.

Area and river of origin of the test organism

This topic falls under the ambit of river waters with different chemical profiles, their subsequent effects on salt speciation, and differences in recorded toxicity responses. It is also relevant if evaluating whether guidelines for managing freshwater salinisation should be developed at national, regional or site-specific level. It is possible that the analysis of toxicity data may indicate all organisms tested fall within the same range of allowable biological variability, but management objectives may require different management strategies for different rivers or different regions. Management objectives will obviously depend on the uses of the river being evaluated.

Stream type and experimental design

Salinity testing at CAT-IWR has been conducted in a number of different stream designs, and using both a replicated and unreplicated regression design. See Binder (1999) for a comprehensive evaluation of the three recirculating artificial stream designs being used at CAT-IWR. The stream design used for a particular experiment is dependent on the objective of the experiment, and practicalities such as space availability. The experimental design used is also

dependent on experimental objectives and factors such as the availability of test organisms. The following extract regarding experimental design is from a methods protocol produced by CAT-IWR staff for conducting toxicity tests using indigenous riverine invertebrates in recirculating artificial streams (DWAF, 2000):

> "A regression design is recommended for acute toxicity testing, as it generates concentration-response information. Replication of test concentrations are often difficult to achieve, and replication may be limited by the numbers of test organisms available. This is particularly important as it is not recommended to run less than six concentrations when trying to determine a concentration-response relationship.
>
> It is assumed that an unreplicated regression approach will be followed for regulatory purposes. ***The unreplicated design can be duplicated to assess experimental variability.***
>
> Where possible, the following procedures and methods are recommended for conducting successful toxicity tests:
>
> A duplicated regression design, with the use of at least six concentrations of the test toxicant, producing at least 4 partial responses. Duplication is considered necessary due to the variability inherent to indigenous organisms. Each regression set will then be analysed separately and the LC50s (for example) compared.
>
> If the toxicant range cannot be duplicated (e.g. due to limited numbers of test organisms), a single regression design can be followed. At least eight concentrations of the toxicant and a control should then be tested, as a minimum of six concentrations are needed to adequately describe a concentration-response relationship (Gerhardt, pers. comm.). This will improve the reliability of the data, and maximise the number of data points on the straight line section of the dose-response curve."

As stream and experimental design are selected on an experiment-by-experiment basis, it is necessary to determine the contribution of these factors to the variability in tolerance data. Although both replicated and regression experimental designs have been used at CAT-IWR, most of the mortality data is unreplicated. This information will therefore be used for variability assessments. An evaluation will also be done for both 4 and 10-day data. The use of longer-term data adds a component of duration to the assessment.

In addition to the above-mentioned factors related to biological variability, the differences between *experimental (analytical)* and *biological variability* must be identified, and acceptable levels of variability for each determined. Information on the test organism itself, i.e. understanding the biology of the organism, will assist in distinguishing between these two sources of variability. Unfortunately little information is available on many South African organisms; this limitation has been acknowledged in other studies (e.g. Jooste et al., 2000).

Now that a protocol of methods has been produced for toxicity testing using indigenous macroinvertebrates (DWAF, 2000), an urgent requirement is an assessment of the acceptability

of toxicity data, i.e. what range of test results would be considered acceptable in terms of a standard method. To achieve this, it is necessary to evaluate toxicity data generated by CAT-IWR against international data, and particularly against data generated using the standard test organism *Daphnia*. See Appendix 2.1 for data used.

Another confounding factor is the differentiation between *statistical* and *biological significance*. Although data may be shown to be statistically significantly different, differences do not always appear to be biologically significant. This phenomenon may be more relevant for system variables, such as salinity, than for toxics.

The aim of this section of Chapter 2 is therefore to generate guidelines for incorporating and identifying variability of toxicity data using indigenous macroinvertebrates in artificial streams, and to set guidelines for the acceptability of toxicity data using these systems. As much of the data in the CAT-IWR toxicity database concerns salinity toxicity and lethality as the end-point, guidelines will be produced based on this data. It is therefore possible that guidelines may be different for other toxicants, e.g. metals, and other biological end-points, particularly as an end-point may be more sensitive to one toxicant than another. End-points and toxicants should therefore be evaluated separately for variability (US EPA, 2000). The data used for this evaluation, and the method of extraction from the CAT database, will be presented in Section 2.1.3 and Appendix 2.1.

2.1.2 Methods available for determining variability

In June 2000 the United States Environmental Protection Agency (US EPA) produced a document on accounting for method variability in whole effluent toxicity (WET) testing (US EPA, 2000). Although this document is specific to WET testing, the principles are valid to this report.

Methods available for determining variability include the following:

- Coefficient of variation (CV)
- Minimum significant difference (MSD)
- Determining statistical differences by graphical displays of confidence intervals.
- Ratio of measurements

Coefficient of variation (CV)

The CV provides a measure of the sample variation relative to the mean, and can be used to compare the variability of two data sets with different means. The CV is therefore simply the standard deviation divided by the mean (Fowler and Cohen, 1993). As a measure of test precision is a measure of the CV (or %CV) of point estimates of a number of tests (Denton and Norberg-King, 1996), it is a more efficient method for determining variability for point estimates than NOECs (US EPA, 2000). CV is considered a suitable method for the evaluation of CAT-IWR toxicity data.

Minimum significant difference (MSD)

The MSD represents the smallest difference between the control mean and a treatment mean that leads to the statistical rejection of the null hypothesis (i.e. no toxicity) at each concentration of the dilution series. It therefore indicates within-test variability and test method sensitivity, is applicable to replicated testing, and has been widely used for evaluating WET test variability (Wang et al., 2000).

Confidence intervals (CIs)

This evaluation of variability is concerned with the overlapping of 95% CIs and statistical significance. When a point (e.g. a LC50 value) is plotted on a graph with its 95% CIs, and the CIs overlap with those of another point on the graph, the two points are said to *not* be significantly different. However, when the CIs do not overlap, the two points *are* statistically significantly different (Everitt, 1999).

Ratio of measurements

Ratios can be used to quantify variability of EC25, EC50 and NOEC measurements (Chapman et al., 1996; US EPA, 2000). For example, the ratio of the largest and smallest EC50 values (the maximum ratio) is calculated per organism type (e.g. species, family) per set of experiments (Binder, 1999).

2.1.3 Method used in this study for assessing experimental variability

Effect concentrations, i.e. EC50 and LC50 values, were used for assessing data variability (see Section 2.1.4), as the least variability in the characteristic sigmoid concentration-response curve is at the 50% level of response (Rand, 1995). Note that this is valid for Probit estimations of mortality, which is the primary statistical method used in this study. Effect concentrations are concentrations of the test material derived from the observed biological end-point, followed by data analysis using either hypothesis testing procedures or point estimate techniques (US EPA, 2000). In this instance, point estimate techniques were generally used to produce EC50 and LC50 values, as much of the data analysed was unreplicated. Where replicates of the same range of toxicant concentrations were run, each set was analysed separately. *Coefficient of variation (CV)* was the method of choice for assessing the variability of results as this method is widely used and appropriate for point estimate data. As mentioned in Section 2.1.1, data analysed was restricted to salinity data with mortality as an end-point. Four-day and 10-day data was analysed where possible. The end-point of the 10-day chronic tests was also mortality. At CAT-IWR, mortality data is analysed for EC50 or LC50 values using either Probit or Spearman-Karber methods. Before this information is included in the assessment of CV, the quality of the data is assessed. Extreme outliers (2 cases only) were not included in the analysis. The assessment was conducted in terms of the following parameters. Please note that *all* parameters must be evaluated to make an accurate assessment of the quality of the data, together with professional judgement.

- χ^2 values - Probit model: These values are an assessment of how accurately the data being analysed fits the statistical model. US EPA version 1.5 produces both χ^2 tabulated and χ^2 calculated values. The general guideline is that if χ^2 calculated > χ^2 tabulated, then

significant heterogeneity exists and data is not normally distributed. This result would not be valid.

- Confidence limits (CLs) - Probit and Spearman-Karber models: Upper and lower 95% confidence limits are produced, which are very useful in assessing data quality. The smaller the CLs around the LC50 or EC50, the more precise the data.
- % trim - Trimmed Spearman-Karber model: This indicates how much data has been trimmed off the extreme edges of the sigmoid concentration-response curve so as to best fit the model. The lower the % trim, the more accurate the data.
- Warning messages - Probit model: A number of warning messages are issued by Probit if the data being analysed does not fit the model accurately. These should be evaluated using professional judgment, as warning messages may not be issued even when the CLs are wide.

2.1.4 Data used for variability assessments

All toxicology information produced by CAT-IWR staff is curated in the CAT toxicology database (see details in Appendix 1) after analysis. A "report form" screen enables the extraction of data in various forms, e.g. Probit data for *Adenophlebia auriculata* from the Palmiet River, Eastern Cape, using sodium sulphate as toxicant, and the channels as experimental system. To test the variability parameters listed in Section 2.1.1, the extractions listed in Appendix 2 were conducted. Please note that all data listed in Appendix 2 were not used for analysis. Only data producing a reliable LC50 or EC50 value were used for calculating CVs. Probit and Spearman-Karber data were extracted with the selected parameters. For $CaSO_4$ and $MgSO_4$ experiments, only cumulative mortality data (the selected biological end-point) were extracted as mortality data was not appropriate for Probit and Spearman-Karber analysis. Due to the quality of this data, these salts were not included in the assessment.

2.1.5 Results

Data used in this assessment were screened and selected according to the parameters listed in Section 2.1.3 before analysis. Results are shown in Table 2.1.1 as %CV. Where data was insufficient for analysis, only means are indicated.

TABLE 2.1
TABLE OF %CVS, MEANS AND NUMBER OF DATA POINTS FOR PARAMETERS IN TABLE 1, APPENDIX 2.1

Parameter		%CV	mean LC50 (min-max) (g/l)	No. of data points
Test organism:	Ephemeroptera + NaCl (4 days) Ephemeroptera + NaCl (10 days)	37.88 34.50	5.54 (1.60-7.90) 2.85 (0.84-5.90)	10 7
Test organism:	Ephemeroptera + Na_2SO_4 (4 days)	52.59	5.00 (1.95-10.30)	12
Test organism: (i.e. NaCl and Na_2SO_4 only) *	Ephemeroptera + salt (4 days)	58.67	5.94 (1.60-10.30)	19
Test organism:	*Tricorythus* + NaCl (4 days)	55.20	3.90 (1.60 - 6.20)	4
Test organism:	*Tricorythus* + NaCl (10 days)	-	3.05	2
Test organism:	*Tricorythus* + Na_2SO_4 (4 days)	33.13	3.73 (1.95 - 5.10)	6
Test organism:	*Tricorythus* + Na_2SO_4 (10 days)	48.56	1.43	4
Test organism:	*Afronurus* + NaCl	-	5.64	2
Test organism:	Ephemeroptera + NaCl (4 days) *Daphnia pulex* + NaCl (2 days)	37.88 20.32	5.54 (1.60-7.90) 3.17 (2.40 - 4.03)	10 7
Test organism:	Ephemeroptera + Na_2SO_4 (4 days) *Caridina nilotica* (adults) + Na_2SO_4 (4 days)	52.59 -	5.00 (1.95-10.30) 4.39	12 2
Area + river:	*Tricorythus* per river + NaCl (4 days)			
	Kat River	-	6.20	1
	Sabie River	-	2.1	2
	Breede River	-	5.10	1
Area + river:	*Euthraulus* per river + NaCl (4 days)			
	Keurbooms River	-	5.91	1
	Vaal River	-	7.4	2
Area + river:	*Euthraulus* per river + NaCl (10 days)			
	Keurbooms River	-	2.21	1
	Kat River	-	3.82	1
Area + river:	*Adenophlebia* per river + NaCl (10 days)			
	Kat River	-	3.82	1
	Palmiet River	-	5.65	2
Area + river:	*Afronurus* per river + NaCl (4 days)			
	Molenaars River	-	4.61	1
	Vaal River	-	6.67	1
Area + river:	*Tricorythus* per river + Na_2SO_4 (4 days)			
	Sabie River	42.67	3.00 (1.95 - 4.43)	3
	Olifants River	17.20	4.46 (3.61 - 5.1)	3
Area + river:	*Tricorythus* per river + Na_2SO_4 (10 days)			
	Sabie River	-	0.43	1
	Olifants River	13.5	1.76 (1.55 - 2.02)	3

TABLE 2.1
TABLE OF %CVS, MEANS AND NUMBER OF DATA POINTS FOR PARAMETERS IN TABLE 1, APPENDIX 2.1

Parameter		%CV	mean LC50 (min-max) (g/l)	No. of data points
Toxicant:	NaCl (Sabie River, *Tricorythus tinctus*, channels, river water) (4 days)	-	2.1	2
	Na_2SO_4 (Sabie River, *Tricorythus tinctus*, channels, river water) (4 days)	42.67	3.00 (1.95 - 4.43)	3
Toxicant:	NaCl (Palmiet River, *Adenophlebia auriculata*, channels, dechlorinated. tap water) (4 days)	-	7.91	1
	Na_2SO_4 (Palmiet River, *Adenophlebia auriculata*, channels, dechlorinated. tap water) (4 days)	19.91	7.54 (6.36 - 9.23)	3
Diluent:	River water (*Daphnia pulex*, NaCl)	-	2.37	2
	Reconstituted tap water (*Daphnia pulex*, NaCl)		2.96	2
Stream type:	*Adenophlebia auriculata* + Na_2SO_4, Palmiet River, dechlorinated tap water			
	- Large artificial stream units	-	8.07	1
	- Raceways	-	10.40	1
	- Channels	19.91	7.54 (6.36 - 9.23)	3
	- all stream types	19.88	8.22 (6.36 - 10.40)	5

*: Calcium and magnesium sulphate data not included in the analysis due to poor data quality.
-: Insufficient data for calculating CVs.

2.1.6 Discussion and conclusion

This section contains an assessment of the results achieved, limitations of the data being evaluated, and a list of generic issues related to evaluating variability.

Variability results

Many of the questions posed in Section 2.1.1 remain unanswered, partly due to the levels of variability shown by the data, and the limitations of the small database of information available for this assessment. In many instances it was not possible to determine %CVs and mean values had to be used. According to Norberg-King (pers. comm. with Everitt, IWR, 1999) a %CV of 20-30% is acceptable for conditions where the same experimental protocol, same water, same organisms, same conditions, same person and same stock solutions are used. In an organisation such as CAT-IWR where results are product-related, this level of repeatable conditions is impossible to achieve.

As expected, experiments using the standard laboratory organism *D. pulex* show lower %CV than when using indigenous invertebrates, with the use of river water vs. reconstituted tap water showing similar results (i.e. means of 2.37 vs. 2.96 respectively). The use of different stream

types also has a low %CV (i.e. 19.88), suggesting that the use of indigenous invertebrates has a greater effect on results. When comparing results of using different salts to increase salinity when all other parameters stay the same, NaCl and Na_2SO_4 show similar results (Sabie River: mean of 2.1 vs. 3.0 for *T. tinctus*, and Palmiet River: mean of 7.9 vs. 7.5 for *A. auriculata*). When also assessing other mayfly results, there does not appear to be a clear pattern relating mayfly data to the salt used for salinisation. Results vary between 33 and 59 %CV.

When comparing area of origin of organisms, rivers generally show similar results, except for the Sabie River. The *Tricorythus* species in this river seems to be particularly sensitive, and would be a useful indicator for management if a protective approach was followed for this river.

Limitations of CAT-IWR data

Although it is recognized that the CAT-IWR salinity database is small for the purposes of assessing variability accurately, addressing the following issues will improve the accuracy of applications of toxicity data.

- Relatively few species have been tested. It is understood that the data produced by CAT-IWR has been strongly linked to contractual obligations and product-based research, but more accurate assessments can obviously be made when more data is available.
- The majority of the data is acute, with some 10-day sub-chronic testing results. Confidence in 10-day data is low due to the size of the database.
- Limited use of reference toxicants. A structured programme must include the use of reference toxicants for accurately assessing variability. This is critical to an effective Quality Assurance programme.
- An effective assessment of variability was difficult due to the gaps in the available data, e.g. data for standard organisms such as *Caridina* (method being developed at CAT-IWR for use as an indigenous standard laboratory organism) and *Daphnia* should be available for both NaCl and Na_2SO_4. This is again linked to the contractual nature of the research.

Statistical tests

A lack of sufficient confidence in the statistical analysis of toxicity test results imply that a statistically non-significant result cannot be taken to imply the *absence* of a real toxic effect. It is therefore very important to consider the validity and reliability of the test results. Current statistical tests may provide inadequate protection against drawing the wrong conclusion when a concentration does have a toxic effect (Shukla, 2000). This is particularly important when evaluating Probit results. As mentioned in Section 2.1.4, there is often a mismatch between warnings issued by the programme and other parameters, e.g. the size of the CLs, which engenders a lack of trust in the results produced by the programme. Although Spearman-Karber produces more unequivocal results, this is clearly related to the robust nature of the programme. In the light of this information, it is important to use professional judgement based on experience, knowledge of what you are trying to assess and knowledge of the system, when evaluating the accuracy of the data produced.

2.1.7 Recommendations

The accuracy of a toxicity test result can obviously be improved by increasing sample size,

decreasing the variability in response, or limiting the analysis to detection of large differences between the treatment and the control (Shukla, 2000). The results of this study were limited due to the small database evaluated, resulting in a lower confidence in the accuracy and reliability of results and recommendations emanating from the study. It is however recognized that this is inherent to the small pool of data being considered, and the lack of being able to conduct inter-laboratory evaluations.

It is recommended that the use of CVs for assessing the variability of CAT-IWR toxicity data - particularly for assessing experimental variability - be included in a Quality Assurance programme. The way in which variability testing is structured will depend on what data is being evaluated and the objective of the evaluation (as shown in this study (Appendix 2.1)). The programme should include an evaluation with previous results, as well as internationally accepted variability parameters and data. It is understood that acceptable variability margins may differ depending on the application of data, e.g. parameters of acceptability for use of data in site-specific Reserve assessments may be different to traditional concentration-response assessments, but acceptable variability values and data quality should be defined for each application. It is also recommended that as the WET testing database expands, variability methods specific to WET testing be evaluated, particularly as variability associated with an effluent is obviously higher than a single substance.

Due to the dependence of an accurate variability assessment on the volume and nature of available data, it is recommended that CAT-IWR staff also rely on other parameters for checking data validity, e.g. the application of appropriate acclimation and control mortalities, as well as the parameters listed in Section 2.1.3.

2.2 APPLICATION OF SALINITY TOLERANCE TEST RESULTS IN ECOLOGICAL RESERVE ASSESSMENTS

2.2.1 Introduction

Palmer and Scherman (2000) developed a hazard-based approach to apply site-specific salinity tolerance data in ecological Reserve assessments. WRC-funded projects have been used to research the development and application of the method in the Olifants (Palmer and Rossouw, 2001) and Breede Rivers. (See Chapter 6 for project links.) The application of the method in the Olifants River Ecological Water Requirements Assessment (OREWRA) is presented in Section 2.2.2, and the integration of tolerance data with biomonitoring and chemical data is presented in Section 2.2.3, where the Olifants, Breede and Thukela River ecological Reserve assessments are cited as case studies.

2.2.2 A hazard-based approach to applying tolerance testing in the development of ecological Reserve evaluations for salinity in the Olifants River

Introduction

Methods for the determination of water quality aspects of an ecological Reserve assessment developed rapidly between 1999 and 2000, particularly with regard to the assessment of salinity (a composite term for the concentrations of ions and salts and usually measured as either total dissolved solids (TDS) or electrical conductivity (EC)). In the RDM (Resource Directed Measures) method of Bath et al. (1999), TDS was listed as a system variable, for which the reference condition was described in terms of the median, and 25th/75th percentiles of TDS/conductivity data collected at a reference site; and the classes for the Present Ecological State (PES) were defined as percentage deviations from the reference condition. Bath et al. (1999) successfully applied this method in the Crocodile River, but when the method was applied to the Sabie River each class description seemed to be too strict (Palmer and Scherman, 2000).

Unlike the Sabie River (Palmer et al., 1996; Goetsch and Palmer, 1997), the Olifants River is heavily impacted with regard to TDS (Palmer and Rossouw, 2001), which is a conservative, multi-variate water quality parameter (many different organic and inorganic ions contribute to electrical conductivity and total dissolved solids). When the Bath et al. (1999) method was applied to reaches in the upper Olifants River, there was a considerable difference between the TDS values of the reference site and most of the other sites, and most of the water quality reaches were therefore classified in the E/F health class for salinity. This again seemed to be misleading as the biomonitoring SASS results indicated lower levels of impact.

We suggest that this discrepancy relates to the nature of biotic responses to elevated salinity. Research over the past five years (Palmer et al., 1996; Binder, 1999; Palmer and Scherman, 2000) has indicated that elevated salinity acts as a slow toxicant, and with both acute (96 hour) and chronic (10 day) responses recorded at concentrations well above those usually experienced in rivers. A review of biotic responses to salinity is given in Binder (1999). However, salinity in parts of the Olifants River is excessively elevated, particularly in winter, and it is important to assess the biotic impact of high salinity in a biologically sensible manner.

An alternative method was therefore developed, which used salt tolerance-test data, rather than a percentage deviation from the reference condition, to link TDS values to health classes (Palmer and Scherman, 2000). This alternative method was applied in the OREWRA study, and the method then formed the basis of a revised RDM method (DWAF, 2001). The method is based on the premise that the tolerances of riverine organisms to salts will primarily be affected by their experience of salts in the catchment over evolutionary time, and may be site - or river-specific.

The South African Water Quality Guidelines (DWAF, 1996) for toxic substances were developed using international tolerance database results. Toxic substance guidelines were based on the LC50 values of all the taxa recorded, e.g. Aldenberg and Slob (1993). (LC50 is the concentration at which each individual in the population has a 50% chance of mortality). There is a quite limited international database on the salt tolerances in freshwater invertebrates (Hart et al., 1990;1991; Lowell et al., 1995). Instead of following the Aldenberg and Slob (1993) method, Palmer and Scherman (2000) used the tolerance results from a single dominant taxon in the river reach under consideration. But instead of the LC50 values, they used the LC1 and LC5 values (concentrations at which each individual in the population has a 1% or 5% chance of mortality), and also the 95% confidence limits around the LC1 and LC5 values, to identify health class-TDS relationships. Concentrations less than the 95% confidence limit of the LC1 or LC of short-term chronic (10 day) and acute (4day) exposure tests were termed "inferred" toxicity levels, and, together with the LC1 and LC5 values, were used instead of the mean LC50 values of many species, to calculate CEV and AEV values.

In the OREWRA study, the Palmer and Scherman (2000) method provided health class-TDS relationships that were remarkably consistent with the health classes indicated using biomonitoring. In addition, in those reaches where the Palmer and Scherman (2000) method for salts indicated a better health class than biomonitoring classes, additional metal toxicity test indicated sources of chemical stress other than salts (Palmer and Rossouw, 2001).

The empirical consonance of the biomonitoring and Palmer and Scherman (2000) method was confirmed in the Breede River study (Rossouw, pers. comm.; Scherman et al., in prep.). Jooste has subsequently produced a preliminary salt-health class relationship using the Aldenberg and Slob method (Jooste pers. comm.). When this has been published, the next step will be to compare the two methods and their applicability.

In this section, the application of the Palmer and Scherman (2000) method in the OREWRA study is presented and discussed. The mayfly *Tricorythus discolor* is one of the dominant filter-feeding, riffle-dwelling macroinvertebrates in the Olifants River. *T. discolor* were collected from a minimally salt-impacted reference site (DWAF water quality monitoring site B1H018). Nymphs were transported to the CAT-IWR artificial stream laboratory, and experimentally exposed to elevated salinities, with the aim of experimentally establishing the concentration-response relationship. Sodium sulphate was selected as the toxicant as we had previously shown that sodium sulphate is more toxic than sodium chloride (Goetsch and Palmer, 1997), and sulphate was a dominant ion in many reaches of the Olifants River (Palmer and Rossouw, 2001).

Study site and methods

More than 2000 *T. discolor* nymphs were collected from a riffle on the farm Middelkraal, near the DWAF water quality monitoring site B1H018 in the upper reaches of the Olifants River. Water chemistry data showed this site was minimally salt-impacted, with salinities rising from reference conditions only in the winter months (Fig. 2.1).

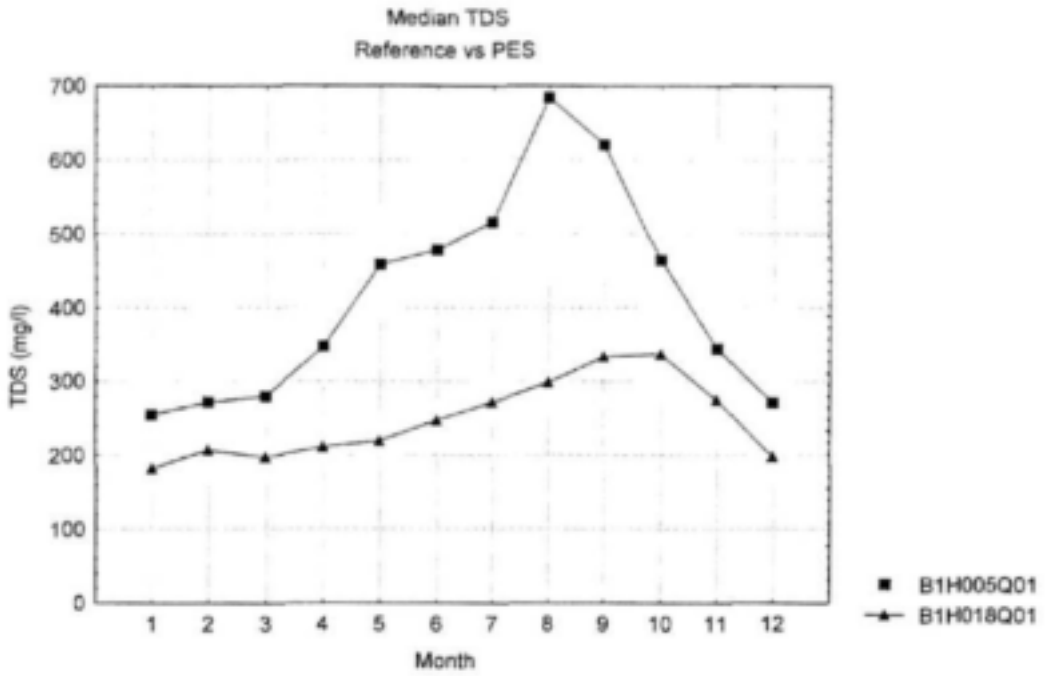

Figure 2.1

The lower line (triangles) shows the median monthly salinities (TDS mg/ℓ) at the site where *Tricorythus discolor* nymphs were collected (DWAF water quality monitoring site B1H018). The upper line (squares) shows the salinities at an impacted site in the upper reaches B1H005. Mayfly nymphs were collected in February when salinity was less than 200 mg/ℓ (30 mS/m).

Individual nymphs were collected off rocks, using a paint brush to avoid damage, and were placed in cooled, aerated river water, in a cooler box. Sponges were placed in the water for the nymphs to cling to. The cooler box, with aerated water and test organisms, was transported by air to the artificial stream laboratory in Grahamstown, where 29 artificial stream channels, each recirculating 20 litres of charcoal-filtered tap-water, had been prepared. Individual nymphs were checked for damage using a dissecting microscope, and between 40 and 50 undamaged nymphs, which were not in the final instar, were placed in each channel. Nymphs were left to acclimate for 48 hours, after which dead individuals were removed. There was less than 1% mortality in each channel, and the experiment was started with the addition of sodium sulphate to fresh filtered tap-water. Three replicate experiments were run with three filtered tap-water controls, and 3 replicates of each of the following concentrations: 0.2, 0.5, 1, 2, 3, 4 and 6 g/ℓ Na_2SO_4.

Channels were checked for mortalities in the morning and evening from the start of the

experiment until the end of Day 10. Fresh salt solutions and controls were made up on Days 4 and 8. Organisms were fed with Tetramin (Haigh and Davies-Coleman, 1997) on Day 5. Full chemical analysis of the test water was conducted by IWQS, DWAF, from water collected from each channel on Days 1, 4, 8, and 10, and temperature, pH and EC (electrical conductivity) were checked daily. Mortality results were analysed using the parametric Probit analysis, and the non-parametric Trimmed Spearman-Karber analysis (APHA, 1992). Both methods yielded similar values. Probit results were used because a variety of *LC* values were provided. (Note: *LC* values estimate the **L**ethal **C**oncentration - this term has been used rather than EC for **E**ffective **C**oncentration even though a surrogate for death - total immobility - was used as the experimental end-point.) All the collection and experimental methods have been described and applied (Palmer et al., 1996; Binder 1999; Palmer and Scherman, 2000; DWAF, 2000).

Results

There were 3 independent sets of mortality results from the Upper Olifants experiments. These were analysed separately to check that the results were within a reasonably similar range (i.e. as a check on the degree of variability in response within the test population), and then the results were pooled so as to take advantage of the large numbers of test organisms used (Table 2.2). The pooled data were used to evaluate the Present Ecological State (PES) for salinity in each water quality resource unit. Probit analysis provided best estimates of the concentrations at which 1% (LC1), 5% (LC5), and 50% (LC50) of the test population died, with their associated 95% confidence limits (Tables 2.3 and 2.4).

TABLE 2.2
LC 1, 5 AND 50 RESULTS OF THE PROBIT ANALYSIS FOR THE INDIVIDUAL EXPERIMENTS AND THE POOLED DATA (CL = CONFIDENCE LIMITS)

LC value	Experiment	Acute (96h)			Chronic (10d)		
		Na_2SO_4 (g/ℓ)	*SO_4 (g/ℓ)*	*EC (mS/m)*	*Na_2SO_4 (g/ℓ)*	*SO_4 (g/ℓ)*	*EC (mS/m)*
LC1	**Pooled data**	**1.07**	**0.72**	**155**	**0.46**	**0.31**	**90**
	Experiment 1	0.9	0.61	145	0.65	0.44	115
	Experiment 2	0.9	0.61	145	0.75	0.51	125
	Experiment 3	1.92	1.30	280	0.34	0.23	75
LC5	**Pooled data**	**1.6**	**1.08**	**235**	**0.68**	**0.46**	**125**
	Experiment 1	1.5	1.01	225	0.84	0.57	140
	Experiment 2	1.38	0.93	205	0.99	0.67	160
	Experiment 3	2.56	1.73	340	0.57	0.39	105
LC50	**Pooled data**	**4.4**	**2.97**	**515**	**1.82**	**1.23**	**265**
	Experiment 1	4.6	3.11	535	1.56	1.05	230
	Experiment 2	3.6	2.43	445	1.72	1.16	250
	Experiment 3	5.11	3.45	5.85	2.02	1.37	285

TABLE 2.3
OLIFANTS RIVER - UPPER REACHERS USING SODIUM SULPHATE: ACUTE TOXICITY RESULTS (96 H) - ESTIMATED LC VALUES AND CONFIDENCE LIMITS (CL)

LC Value	Concentration (g/ℓ) Na_2SO_4	Lower 95% CL	Upper 95% CL	EC mS/m (Na_2SO_4)	Lower 95% CL	Upper 95% CL
LC1	1.08	0.29	1.71	155	65	250
LC5	1.63	0.65	2.29	235	110	315
LC50	4.45	3.69	5.59	515	445	625

TABLE 2.4

OLIFANTS RIVER - UPPER REACHES USING SODIUM SULPHATE: CHRONIC TOXICITY RESULTS (DAY 10) - ESTIMATED LC VALUES AND CONFIDENCE LIMITS (CL)

LC Value	Concentration (g/ℓ) Na_2SO_4	Lower 95% CL	Upper 95% CL	EC mS/m (Na_2SO_4)	Lower 95% CL	Upper 95% CL
LC1	0.49	0.002	1.06	90	30	165
LC5	0.72	0.009	1.34	125	45	200
LC50	1.82	0.46	2.65	265	90	355

These LC values are used, as described in Palmer and Scherman, (2000) to calculate the AEV and CEV values (Roux et al., 1996; Tables 2.5 and 2.6).

TABLE 2.5

CALCULATIONS OF POSSIBLE ACUTE EFFECTS VALUE (AEV), AND CHRONIC EFFECTS VALUE (CEV) (DWAF, 1996) USING SALINITY TOLERANCE TEST RESULTS. MAYFLY (*TRICORYTHUS DISCOLOR*) NYMPHS WERE EXPOSED TO SODIUM SULPHATE. THE HAZARD-BASED USE OF LC1 AND LC5 VALUES IS DESCRIBED MORE FULLY IN PALMER AND SCHERMAN (2000), AND THE VALUES IN BOLD ARE USED IN TABLE 2.6 AND/OR 2.7

Note : Although test results from only one taxon were used, the safety factors designated in DWAF (1996) have not been applied because LC1 (with a 1% mortality risk) and LC5 (with a 5% mortality risk), rather than the mean LC50 values from a range of taxa, were used.

Acute Effects Value (AEV)

1. Calculate the Final Acute Value (FAV) - use the LC5* or LC1* for acute tests:

4 Day exposure	Na_2SO_4 (mS/m)
LC5*	**235**
LC1*	**155**

2. Calculate the Acute effects Value (AEV)

FAV / 2		Na_2SO_4 (mS/m)
	LC5*	**118**
	LC1*	**78**

Chronic Effects Value (CEV)

CEV can be calculated in 2 ways:

A. Use the LC5* or LC1* for chronic tests:

	Na_2SO_4 (mS/m)
LC5* for chronic tests:	**125**
LC1* for chronic tests:	**90**

B. 1. Calculate the acute:chronic ratio (ACR) - $\frac{\text{acute LC50}}{\text{chronic LC50}} = \frac{515}{265} = 1.94$

2. CEV = $\frac{\text{FAV}}{\text{ACR}}$

	Na_2SO_4 (mS/m)
LC5*	**121**
LC1*	**80**

TABLE 2.6
ACUTE EFFECTS VALUE (AEV) AND CHRONIC EFFECTS VALUE (CEV) CALCULATED FROM LC1 AND LC5 VALUES (mS/m)

LC5 - based AEV	118	LC5 - based CEV	125
LC1 - based AEV	78	LC1 - based CEV	90
		LC5 - based CEV - using the acute to chronic ratio calculation	121
		LC1 - based CEV - using the acute to chronic ratio calculation	80

The LC, AEV and CEV values were then used to derive a ranked hazard table (Table 2.7), and Table 2.8 indicates the hazard descriptions associated with these ranked tolerance end points.

TABLE 2.7
LIST OF CLASSES AND TOLERANCE END-POINTS WITH THE ASSOCIATED CONDUCTIVITY; RANKED BY INCREASING HAZARD POSED TO THE MAYFLY *Tricorythus discolor*, FROM EXPOSURE TO SODIUM SULPHATE SOLUTIONS. WHERE: ACUTE = 4-DAY EXPOSURE, CHRONIC = 10-DAY EXPOSURE, H= HAZARD SEQUENCE, AND * INDICATES 30MS/M - THE ANNUAL MEDIAN SALINITY FOR THE REFERENCE SITE (SEE FIGURE 2.1)

Sodium Sulphate (Na_2SO_4)			
H	***Tolerance test result***	***Electrical conductivity mS/m***	***Class***
1*	Annual median salinity for the Upper Olifants reference and test organism collection site, and below the lower 95% confidence limit of the chronic LC1	30	A
2	The lower 95% confidence limit of the chronic LC5	45	B
4	LC1 - based AEV	78	
5	LC1 - based CEV - using the acute to chronic ratio calculation	80	
6	The lower 95% confidence limit of the chronic LC50 and Chronic LC1	90	D
7	The lower 95% confidence limit of the acute LC5	110	
8	LC5 - based AEV	118	
9	LC5 - based CEV - using the acute to chronic ratio calculation	121	
10	Chronic LC5	125	E
11	Acute LC1	155	F
12	Acute LC5	235	

TABLE 2.8

EXAMPLES OF SELECTED TOLERANCE END-POINTS AND ASSOCIATED HAZARD DESCRIPTIONS. SIMILAR DESCRIPTIONS CAN BE DERIVED FOR ANY LC VALUE AND ASSOCIATED CONFIDENCE LIMITS. OF THOSE LISTED HERE, THE LC50 IS THE MOST ACCURATE, AND THE *LC5* AND *LC1* INDICATE CONCENTRATIONS POSING A LOWER HAZARD. ACUTE EXPOSURE : 4DAYS; SHORT-TERM CHRONIC EXPOSURE: 10 DAYS; ALL END-POINTS: MORTALITY

Tolerance end-point	Risk description
Below the low 95% confidence limit for the LC1	concentrations at which each nymph has <1% chance of mortality
Below the low 95% confidence limit for the LC5	concentrations at which each nymph has <5% chance of mortality
LC1	best estimate of concentration where each nymph has 1% chance of mortality
LC5	best estimate of concentration where each nymph has 5% chance of mortality
LC50	best estimate of concentration where each nymph has 50% chance of mortality
AEV	A measurable acute effect - may be associated with 1% (LC1) or 5% (LC5) risk of mortality
CEV	A measurable chronic effect - may be associated with 1% (LC1) or 5% (LC5) risk of mortality

Table 2.9 provides at risk-based site-specific salinity guideline, related to health classification, for the upper Olifants River.

TABLE 2.9

A HAZARD-BASED, SITE-SPECIFIC SALINITY GUIDELINE FOR THE UPPER REACHES OF THE OLIFANTS RIVER, FOR HEALTH CLASSES A-F. THE RANGES GIVEN INDICATE THE MEDIAN INSTREAM SALINITY THAT SHOULD NOT BE EXCEEDED IN THE LOWEST FLOW MONTH. CLASSES E AND F ARE INDICATED BY SALINITIES WHICH WOULD RESULT IN BIOTIC DEGRADATION. THE WATER QUALITY OBJECTIVE FOR EACH CLASS IS GIVEN (PALMER, 1999)

Class A	**20 - 30 mS/m**
Water quality	Unmodified. Allow minimal risk to sensitive species. Remain within the target water quality range (TWQR, sensu DWAF 1996).
Risk	*Within this range each nymph would face a less than 1% risk of mortality from exposures of up to 10 days.*
Class B	**30 - 45 mS/m**
Water quality	Use Aquatic Ecosystems guideline values (DWAF, 1996) such as chronic effects value (CEV) and TWQR to set objectives that pose slight risk to intolerant organisms.
Risk	*Within this range each nymph would face a less than 5% risk of mortality from exposures of up to 10 days.*
Class C	**45 - 80 mS/m**
Water quality	Use aquatic ecosystems guideline values such as Acute Effects Values (AEV), CEV, and TWQR to set objectives that allow moderate risk only to intolerant biota.
Risk	*Within this range there is a risk of 1-5% mortality from exposures of longer than 10 days, and 1% mortality from exposures of more than 4 days.*
Class D	**80 - 120 mS/m**
Water quality	Use aquatic ecosystem guidelines values (AEV,CEV<TWQR) to set objective that may result in high risk to intolerant biota.
Risk	*Within this range falls the best estimate of that 1% mortality would occur, but that less than 5% mortality would occur, from exposures of longer than 4 days. In addition, 5% mortality would be expected, and up to 50% mortality could occur, from exposures of longer than 10 days.*
Class E	**120 - 155 mS/m**
Water quality	The water quality poses the risk of ecological degradation. The minimum management objective should be those for a D class.
Risk	*Within this range there is the best estimate the 5% mortality would occur after exposures of longer than 10 days.*
Class F	**> 155 mS/m**
Risk	*Above 155 mS/m mortality would be expected to occur after short term (4day) exposure.*

Tables 2.10i and 2.10ii show a comparison of the salinity class ranges in the Sabie and Olifants Rivers. The results of similar tolerance testing from the same genus of mayfly from the Sabie River (Palmer and Scherman, 2000) were used to assess the Blyde River since it has naturally lower salinities, that are more similar to the Sabie River than to the main-stem of the Olifants River.

TABLE 2.10i
RELATIONSHIP BETWEEN RIVER CLASS AND SALINITY FOR ALL RIVER REACHES IN THE OLIFANTS STUDY AREA EXCEPT THE BLYDE RIVER

Assessment class	Electrical conductivity (mS/m)	Total dissolved solids (mg/ℓ)
A	20-35	130-195
B	35 - 45	195-293
C	45 - 80	293-520
D	80 - 120	520 - 780
E	120-155	780-1020
F	>155	>1020

TABLE 2.10ii
RELATIONSHIP BETWEEN RIVER CLASS AND SALINITY FOR THE BLYDE RIVER BASED ON SABIE RIVER DATA

Assessment class	Electrical conductivity (mS/m)	Total dissolved solids (mg/ℓ)
A	8-17	50-100
B	17-30	100-195
C	30-40	195-260
D	40-50	260-325
E/F	>50	>325

Discussion

Until the Palmer and Scherman (2000) method is published in the peer-reviewed journal literature, the method should be regarded as preliminary and exploratory. It has been presented at conferences in South Africa and Australia and was well received. At this stage there are a number of important points to be made:

- The same approach, using organisms of the same genus, in two rivers (Sabie and Olifants) with very different natural and impacted salinity profiles, produced results which were consistent with each river's assessed ecological condition, and biomonitoring

results; and

- the approach gave a much better assessment of salinity than the Bath et al. (1999) method for both rivers, with degraded reaches in the Olifants successfully identified and discriminated from less impacted reaches.

2.2.3 Application of the tolerance results within the OREWRA water quality Reserve assessment

The overall method for the intermediate determination of ecological Reserve consisted of the following seven steps (DWAF, 2000):

(1) Delineate the geographic boundaries of the resource,
(2) determine the water quality river reaches of the resource,
(3) determine reference conditions,
(4) assess the present ecological state (PES),
(5) select a future management class,
(6) assign the ecological Reserve, and
(7) design a monitoring system.

The tolerance data were applied in Steps 3 and 4, where the reference condition and the PES were assessed.

Reference conditions

The *water quality* reference condition is used to describe the natural unimpacted characteristics of a particular section of a river course and to describe the seasonal variation in the data. Reference conditions were determined for:

- *System variables* - Total dissolved solids (TDS), pH, dissolved oxygen (DO), suspended solids and water temperature;
- *Nutrients* - soluble phosphorus and N:P ratio, and
- *Toxic chemicals* - ammonia, organics, inorganics and trace metals.

For each of the system variables, a time series plot was prepared and the data record was examined for positive or negative trends. If a trend existed, the most unmodified ("best") part of the water quality record was selected as the reference condition. Where no trend existed, the most recent five years of data were used. The monthly 25th, 50th and 75th percentiles were then calculated for each of the system variables. For salinity, class assessments were based on tolerance experiments as described in Section 2.2.2, and were equivalent to Class A.

For the nutrients phosphorus and nitrogen it was assumed that the reference condition would be specified by the limits for a Class A river (DWAF, 2000c).

Toxic substances are assumed to be those specified in the South African Water Quality Guidelines (DWAF, 1996). For toxic organic and inorganic compounds and trace metals, it was assumed that the reference condition is specified by Class A limits (DWAF, 2000c) which are the target water quality ranges (TWQR) specified in the SA Water Quality Guidelines (DWAF, 1996).

Present Ecological State (PES)

The Present Ecological State is used to describe the current water quality conditions in the specified river reach. The most recent three to five years of data were used depending on the number of observations in the data set. In cases where samples were collected infrequently, longer data periods than the previous 5 years were used.

The PES class assessments for TDS (Tables 2.10i and 2.10ii) were based on the experimental salinity tolerance results and the results of similar tolerance testing from the same genus of mayfly from the Sabie River (Palmer and Scherman, 2000) which were used to assess the Blyde River. (The Blyde River has naturally lower salinities, that are more similar to the Sabie River than to the main-stem of the Olifants River.)

For the other system variables, an assessment class was assigned based on a *percentage difference* between the reference condition and the present status, using tables from DWAF (1999). Nutrients were evaluated against tables from DWAF (1999).

Evaluation of toxic substances proved to be difficult. There is no routine monitoring for toxic substances in South Africa. Although the river health class indicated by the salinity tolerance tests for each river reach was usually consistent with the biomonitoring classes, there were reaches where the salinity class indicated a better state of health than the biomonitoring. This was taken as an indication that some water quality variable other than salinity was affecting the biota. In each if these reaches a limited instream toxicity test-series was undertaken, which showed two sites with measurable, acute instream toxicity (Palmer and Rossouw, (Appendix 2) 2001). This result emphasizes the importance of regular toxicity monitoring. The restricted nature of the instream toxicity testing limited the PES assessment of water quality in the Olifants River.

The toxicity report (Palmer and Rossouw, (Appendix 2) 2001) drew attention to the fact that chronic tests are more sensitive, and may have detected other sites with instream toxicity. A negative result - not finding acute instream toxicity - did not mean toxicity was not a problem, and finding two incidences of acute instream toxicity, in a single sample set, indicates it could easily be more prevalent. If regular instream toxicity testing were undertaken, the frequency of measurable instream toxicity might be shown to be acceptably low, but in the absence of such data, measurable instream toxicity was taken to indicate ecosystem degradation.

Classification procedure

In addition to the detailed status descriptions of each reach, the Present Ecological State (PES) is finally presented as a river health class. Arriving at a class for water quality is a complex procedure as water quality comprises many water chemistry variables.

The RDM Methodology (DWAF, 2000c) does not give guidance on calculating an integrated PES class for the water quality of a specific reach. The OREWRA water quality project team developed a procedure for summarising the monthly classes and for integrating across the variables to calculate an overall water quality class (Palmer and Rossouw, 2001). This procedure was followed in the OREWRA study, has been submitted to, and is currently under review, by the DWAF RDM team.

2.2.4 Links between ecotoxicology, biomonitoring and water chemistry in the integration of water quality into environmental flow assessments

The content of this section has been submitted for publication to *Rivers Research and Application.*

Introduction

The South African National Water Act (No. 36 of 1998) provides for the ecological Reserve, defined as the quantity and quality of water necessary to protect aquatic ecosystems, in order to ensure ecological sustainability while still allowing use of the resource. In ecological Reserve assessments, the quantity and quality of water, as well as the patterns of flow and concentrations of water quality constituents, are determined and qualitative and quantitative Resource Quality Objectives (RQO) set for water quality, water quantity, habitat and biota (Jooste et al., 2000). These RQOs are recommended in order to meet the flow and chemical concentration needs of ecosystem components such as biota (invertebrates, fish, riparian and in-stream vegetation) and habitat (hydraulics and geomorphology), and are monitored using these components. RQOs are linked to different levels of protection (classes) with increasing risk of serious or critical damage to the ecosystem and concomitant loss of ecological sustainability. Ecological Reserve assessments can be undertaken at different levels of increasing complexity, with each level requiring more detailed investigations, data collection and analysis, and resulting, inevitably, in increased confidence in the outcome of the assessment.

Methods for environmental flow assessments developed in response to concern over increased degradation of river health as a result of increased manipulation of river flow (King et al., 2000). As flow regime is a major component of the riverine environment it is hardly surprising that significant research effort has focussed on the impacts of altered flow regime on ecosystem health and integrity (Norris and Thoms, 1999). Flow assessments, and the development of methods for these assessments, have focussed principally on the quantity of water required to maintain ecosystem integrity, while methods for the assessment of water quality have not developed at an equal pace. However, freshwater resources are threatened by pollution world-wide, particularly in arid regions. If the recommended environmental flows are less than present-day flows, and if land-use impacts, discharges and non-point sources of pollution are not addressed at source, reduced flows will result in increased instream concentrations. Flow and hydraulic habitats may be intact, but ecosystem function may be impaired because of the loss, or reduced fitness, of species.

Although a number of water quality models exist, there are to date no complex models that relate changing water quality conditions to an altered and reduced flow regime. However, if the NWA of SA is to be implemented, there is a requirement for methods for providing qualitative and quantitative RQOs for a suite of water quality variables. Malan and Day (in prep.) have developed a simple flow-concentration model for use in rivers, specifically to feed into ecological reserve assessments. This section describes a method to use water quality information, biological assessment information (SASS; South African Scoring System; Chutter, 1998) and toxicological data in an attempt to provide a more ecologically relevant and predictive input to assess the water quality component of environmental flow assessments.

There are limitations to each of the data sources. Chemical data are usually for monthly samples collected at sampling points that have more to do with sampling convenience than imparting ecological understanding. Water quality data modelling and interpolation is much more complex and less developed than hydrological modelling. The need for site-specific information has been emphasized, particularly as aquatic resources display natural differences and anthropogenic changes are superimposed on these natural differences (Norris and Thoms, 1999). In South Africa, catchments are known to have differences in their natural water chemistry (Dallas et al., 1994) and organisms in those water resources have adapted to those particular water quality conditions. It therefore appears that RQOs for water quality for one catchment may not be appropriate for another catchment as a result of the natural differences. Site-specific water quality data do, however, provide some information on variability and trends, and analysis of these data are an important first step. Because of the relatively low frequency (monthly) and low range of variables analysed, the data may indicate that conditions are more ecologically favourable than is the reality.

Therefore the next step is to analyse and integrate invertebrate biomonitoring data which uses the presence of taxa to provide a biologically integrated picture of water quality. There are several rapid bioassessment protocols available (Uys, 1994; Uys et al., 1996; Chutter, 1998). While these biological assessments are useful indicators, they are merely "red flags" indicating a change in conditions. Unless ecotoxicological experiments are undertaken it is not possible to predict to which environmental stressor/s organisms are responding (Carlisle and Clements, 1999).

Ecotoxicity results related biological responses (or tolerances) to physico-chemical values. The information provided by toxicity tests is therefore a useful link between water quality data and biomonitoring data as it provides information on the concentrations of chemicals at which organisms are affected.

The following approach was developed so that water quality assessments could be included in environmental flow assessment and ecological Reserve studies undertaken for the Olifants, Breede and Thukela River catchments, respectively.

The Olifants River (Gauteng, Mpumalanga) is in the north-eastern part of South Africa (Fig. 2.2) and land-use in the catchment is predominantly agricultural, industrial and mining. As a result, water quality issues in the catchment are dominated by sulphate salinization. Data for the Olifants River study was generated during the Olifants River Ecological Water Requirements Assessment (OREWRA), undertaken from 1999 – 2001 for the Department of Water Affairs and Forestry (DWAF).

The Breede River is in the Western Cape (Fig. 2.2) and the catchment is characterised by agricultural land-use; consequently, water quality issues in this catchment are driven by agricultural salinization (sodium chloride). Data was generated during the Breede River Basin Study, undertaken from 2000 – 2001 for DWAF. Water quality investigations were conducted as part of the BBM (Building Block Methodology; King et al., 2000) and DRIFT (Downstream Response to Imposed Flow Transformations; King et al., in press) process for determining the ecological Reserve for water quantity. Water quality issues were not investigated as a separate study although similar data is collected per identified IFR (In-stream Flow Requirements) site as during an ecological Reserve assessment for water quality.

The Thukela River is in KwaZulu-Natal (Fig. 2.2) where the catchment land-use is characterised by mining in the upper reaches of two of the major tributaries, with the remainder of the catchment being characterised by agriculture. Water quality issues are driven by nutrients and agricultural land-use. This study was initiated in 2001 for DWAF; data for this project was generated during the Thukela Water Project decision support phase.

Figure 2.2
Map showing positions of the three catchments (Olifants River, Breede River and Thukela River) used in the development of the water quality model integrating water chemistry, SASS and toxicological data.

Methods

Details of the processes which need to be initiated to undertake an ecological Reserve assessment for water quality are described in Palmer et al. (in prep.) and Malan and Day (in prep.) and only steps relevant to the use of physico-chemical, toxicological and biomonitoring data are mentioned here (Figure 2.3). Note that method development is a continual process for both the biomonitoring and toxicological methods.

Step 1	The Resource Directed Measures documents (DWAF, 1999) describe the procedure for determining Reference Condition (RC) and Present Ecological State (PES) per water quality variable, and per allocated resource unit in the specified study area. This information is integrated following the procedure developed during the Olifants River study (Palmer and Rossouw, 2001) to generate a PES per resource unit for water quality.
Step 2	The trajectory of change is established in the short (5 years) and long term (20 years), and the Ecological Reserve class (ERC) for water quality defined per resource unit.
Step 3	At the ERC specialist meeting, the ERCs per variable (i.e. water quality, geomorphology, riparian vegetation, fish and aquatic invertebrates) are integrated into a single ERC for each RU.

Figure 2.3

The generic procedure for assessing water quality Ecological Reserve classes, at a detailed level (e.g. Olifants and Thukela case studies) is described in Palmer and Rossouw (2001). Only steps relevant to the use of physico-chemical, toxicological and biomonitoring data are shown here. The procedure for determining present ecological state for TDS / EC as part of Step 1 is considered more closely in Figure 2.4.

Analysis of water chemistry data

Water quality data were obtained from the DWAF Hydrological Information Systems (HIS) database for all available monitoring points in each of the catchments and guidelines in the DWAF document "*Resource Directed Measures for Protection of Water Resources: River Ecosystems*" (1999; RDM manual) were used as a starting point for an assessment of water quality in each catchment. Data sets were evaluated for suitability: the RDM manual specifies minimum data requirements in order to successfully undertake a water quality assessment (DWAF, 1999).

Temporal trend analysis of as many of the selected water quality variables recommended by the RDM manual as possible, e.g. system variables (such as TDS/EC and pH), nutrients (such as nitrates) and toxic substances (such as pesticides and metals), is undertaken to determine whether there has been a change in water quality over time (DWAF, 1999). These also allow for an assessment of water quality reference (natural) conditions (RC) and present ecological state (PES). Median and quartile monthly data are then used to assess whether there are seasonal water quality trends which may be flow (or run-off) related for present state conditions. Water quality data from as many monitoring points as possible should be analysed in order to allow for identification of appropriate water quality reference conditions and present ecological state assessments as well as the identification of water quality problem areas. Analysis of separate water quality parameters provides separate water quality classes for each of the parameters which are then integrated into a final water quality class for the resource unit (see Step 1, Fig. 2.3).

Biomonitoring assessments

Biological monitoring data (specifically SASS data; Chutter, 1998) at, or near, water quality monitoring points are obtained from existing databases where possible, or any other data available for the catchment (additional data may be collected if necessary). An invertebrate specialist relates SASS scores to river health classes. SASS data are usually accompanied by limited water quality measurements (EC, TDS, DO, temperature, pH), which can further aid interpretation of information. It is important to note that the way in which SASS information is collected may vary from one practitioner to the next. For example, the data collected by Dickens et al. for the Thukela catchment was by means of a modified and more intensive SASS technique and laboratory identification of samples. The data collected for the Breede project by Dallas was specifically according to SASS methodology. These issues are being addressed by the Quality Assurance module of the South African River Health Programme, but should be flagged when using SASS biomonitoring data as SASS results can vary depending on data collection methods.

Toxicological assessments

Toxicity tests were undertaken to define a concentration-response relationship between a driving water quality parameter and ecosystem health (Hart et al., 1999). In each of these studies, the selected water quality parameter was salinity (TDS/EC; using either sodium sulphate or sodium chloride as toxicant) as this is a system variable regulating essential ecosystem processes (Palmer and Scherman, 2000). In addition, TDS is a good reflection of water quality consequences of the natural geology of an area. Results are used to define classes A to E (Table 2.11) based on Total Dissolved Solids (TDS) and Electrical Conductivity (EC). Recently, classes were redefined (Table 2.11), but for the purposes of this section, the classes A-F will be used.

TABLE 2.11
ALTERNATIVE DESCRIPTIONS OF RIVER HEALTH

Ecological Condition Classes	Ecological Management Classes
A	Natural
B	Good (A/B, B, B/C)
C D	Fair (C, C/D, D)
E F	Poor

Acute (96-hour) and short-term sub-lethal (10-day) toxicology experiments were undertaken using site-specific indigenous invertebrates following a standard protocol (DWAF, 2000). Indigenous invertebrates were collected from reference sites (or least impacted sites) in each of the three study catchments and experiments were undertaken in the catchment where possible (Breede River catchment) or at the Centre for Aquatic Toxicology, Institute for Water Research (CAT-IWR, Grahamstown). Where experiments were undertaken in the catchment (Breede River study) river water was used as diluent, which more effectively approximates environmental realism. Experiments undertaken at CAT-IWR laboratories used dechlorinated tap water as experimental diluent due to practicalities of transporting river water to the laboratory. Toxicants

were selected on the basis of the primary land-use activity in the catchment and was either sodium sulphate (to represent mining land-use) or sodium chloride (as a surrogate for agricultural land-use). Experimental details are summarised in Table 2.12.

TABLE 2.12
DETAILS OF TOXICOLOGY EXPERIMENTS UNDERTAKEN IN THE DEVELOPMENT AND CONTINUED REFINEMENT OF ASSESSING THE WATER QUALITY RESERVE

Catchment	River	Organism	Diluent	Toxicant
Olifants	Olifants River	*Tricorythus discolor* (Tricorythidae)	Dechlorinated tap water	Sodium sulphate
Breede	Molenaars River	*Afronurus barnardi* (Heptageniidae)	River water	Sodium chloride
	Breede River	*Tricorythus discolor* (Tricorythidae)	River water	Sodium chloride
Thukela	Bushmans River (Upper catchment)	*Euthraulus elegans* (Leptophlebiidae) *Afronurus peringueyi* (Heptageniidae)	Dechlorinated tapwater	Sodium chloride
	Mooi River (Lower catchment)	*Euthraulus elegans* (Leptophlebiidae) *Tricorythus discolor* (Tricorythidae)	Dechlorinated tapwater	Sodium chloride

The toxicological data are used to provide the boundary levels for the different classes for environmental flow assessments and are defined in Table 2.13. It must be remembered that toxicity tests undertaken for the purposes of these studies measure an extreme biological end-point, i.e. mortality, and are of relatively short duration (10 days). For this reason, the lower confidence intervals of LC1 of sub-lethal exposures (10-day exposures) are used to define class boundaries. This is in line with DWAFs protective approach to aquatic resources. The way in which toxicity data can be used most effectively in water resource management is currently under discussion (Jooste, pers. comm.).

TABLE 2.13
CLASS BOUNDARIES DEFINED BY TOXICOLOGICAL DATA. THIS TABLE IS USED AS A GUIDELINE AND PROFESSIONAL JUDGEMENT IS ALSO USED TO SET BOUNDARIES

Class	Boundary descriptor (maximum allowable level)
A	Reference condition (natural state) or below measurable short-term chronic toxicity*, i.e. < lower 95% confidence limit of the short-term chronic LC_1
B	Inferred short-term chronic toxicity, i.e. the lower confidence limit of the short-term chronic LC_1
C	Measured short-term chronic toxicity, i.e. short-term LC_1 or LC_5
D	Measured acute toxicity, i.e. acute LC_1 or AEV
E/F	Greater than acute LC_1

* short-term chronic = 10 days (Rand, 1995)

Integrating water chemistry, toxicology and biomonitoring

Ecological Reserve assessments for the three catchments allowed the development of an integrated method (Fig. 2.4) for the use of physico-chemical, biomonitoring and toxicity test results to classify river health condition. The SASS invertebrate data, together with the water quality data, are used to assess whether the boundary levels set using the toxicity data are reasonable: if biomonitoring data suggests that organisms at a particular site are more robust (i.e. can tolerate poorer water quality) or more sensitive (i.e. groups of organisms are absent even though toxicity data suggest they should be present) the boundaries for the TDS classes based on toxicity data can be adjusted. This is justified by the fact that toxicity test data are usually for a limited number of species (one, or at most two, species).

Step 1	Set classes for TDS using toxicological data (for example, Table 2.13) per resource unit (e.g. Olifants and Thukela studies) or IFR site (e.g. Breede River Basin Study).
Step 2	Plot "TDS/EC toxicology classes" on a map of the study area, e.g. Figure 2.5.
Step 3	Plot SASS data and classes on a map of the study area, e.g. Figure 2.5.
Step 4	If there is a discrepancy between "toxicology classes" and SASS allocated classes, interrogate the following data to find the cause: • habitat information (HAM) • other water quality variables, pollution sources and land-use impacts • flow information and dam operational rules • toxics and possible pesticide use
Step 5	If the SASS class is higher than the "TDS/EC toxicology class", and no reason for the discrepancy can be identified, adjust the upper values for the class boundaries. If the SASS class is lower than the "TDS/EC toxicology class", check for possible in-stream toxicity.
Step 6	Assign PES for TDS per resource unit, based on the checked and corrected "TDS/EC toxicology class" table (Table 2.15).
Step 7	For other water quality variable, use summary statistics, box+whisker and scatter plots to evaluate the data. Set PES per variable according to the RDM documents (DWAF, 1999).
Step 8	Water quality information is integrated following the method of DWAF (2000) to generate a PES per resource unit for water quality.

Figure 2.4
The use of toxicological and biomonitoring SASS data for defining the PES for TDS/EC (HAM; Habitat Assessment Matrix).

Results

The method (Fig. 2.4) was developed while determining the water quality component of the Olifants, Breede and Thukela Reserve studies. An attempt is made to explain the context of the water quality assessment for each study, and how the method developed can be most useful. The method shows how chemical, toxicological and biomonitoring data (specifically SASS invertebrate data) can be used in a water quality reserve assessment. Figure 2.3 shows the generic procedure followed, while Figure 2.4 elaborates on Step 1 of Figure 2.3. Tables 2.14 to 2.17 and Figure 2.5 demonstrate the application of the model using the Breede River Basin Study as a case study.

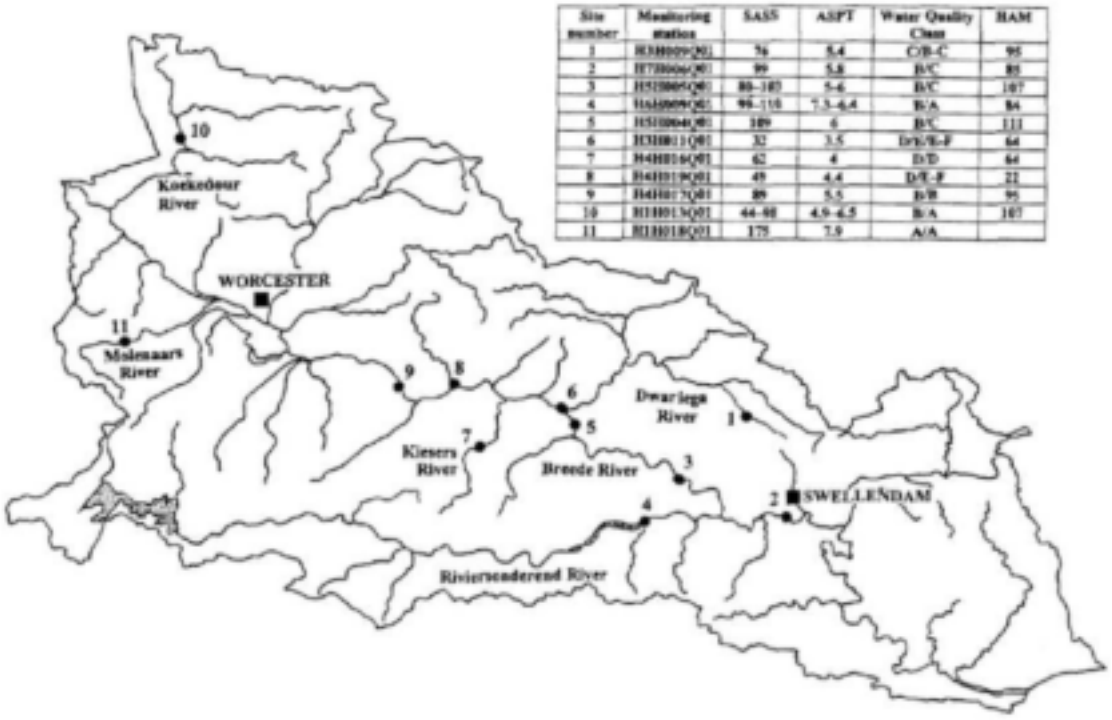

Figure 2.5
Map of the Breede River study area, depicting gauging weirs of interest, together with SASS, ASPT and habitat (HAM) scores, SASS classes and TDS / EC toxicology classes.

TABLE 2.14
TOXICITY DATA FOR THE BREEDE RIVER BASIN STUDY. RESULTS SHOWN ARE CUMULATIVE FOR THE EXPERIMENTAL ORGANISMS TESTED FROM THE BREEDE AND MOLENAARS RIVERS

Toxicity end-point descriptor	TDS (mg/*l*)	EC (mS/m)
Reference	< 45	< 7
Chronic LC_1 lcl*	160	25
Inferred chronic effect / Chronic LC_5 lcl	300	45
Chronic LC_1	500	75
Chronic LC_5	800	120
LC_1 AEV	850	130
Inferred acute effect / Acute LC_1 lcl	1000	150
LC_5 AEV	1100	170
Acute LC_5 lcl	1400	200
Acute LC_1	1700	260
Acute LC_5	2200	330
Chronic LC_{50}	6800	1030
Chronic LC_{85}	9200	1400

lcl: lower confidence limit
AEV: Acute Effects Value (DWAF, 1996) as applied in Palmer and Scherman (2000)

TABLE 2.15
HEALTH CLASS ALLOCATIONS FOR TDS / EC BASED ON TOXICITY DATA (TABLE 2.14)

Class	TDS (mg/*l*)	EC (mS/m)
A	< 45	< 7
B	45 – 300	7 – 45
C	300 – 800	45 – 120
D	800 – 1400	120 – 200
E	1400 – 1700	200 – 260
F	1700 – 2000	> 260

TABLE 2.16

A COMPARISON OF CLASSES OBTAINED FOR SITE-SPECIFIC SASS AND TOXICOLOGICAL ASSESSMENT DATA FOR THE BREEDE RIVER STUDY (SEE FIGURE 2.5 FOR POSITION OF SITES). (NUMBERS IN PARENTHESES REFER TO THE DWAF HIS MONITORING POINTS)

Monitoring site	SASS class	TDS class	Comment
Koekedou River (H1H013Q01)	B/C	A	Habitat good and SASS data carries high confidence therefore agreed to a B class.
Molenaars River (H1H018Q01) IFR Site 2	A	A	Data agreed to an A class.
Breede River (H4H017Q01) IFR Site 3: Le Chasseur	B/C	B	Data agreed to a B class.
Riviersonderend (H6H009Q01)	A/B	B/C	Some debate on the upper boundary of a B class, but on the basis of the SASS data it was retained at 300mg/*l* (Tables 2.15 and 2.17)
Dwariega River (H3H009Q01)	C	B/C	Confirmed the lower boundary for a C class
Breede River (H5H004Q01) Wolvendrift	B	C	Confirmed lower and upper boundaries for a C class (Tables 2.15 and 2.17)
Breede River (H5H005Q01) Wagenboomsheuwel	B	C	TDS data indicated the accuracy of the upper C class boundary
Breede River (H7H006Q01) Swellendam	B	C	An adjustment of the upper C class boundary to 600mg/*l* was considered but rejected in light of data from H5H005Q01
Keisers River (H4H016Q01)	D	D	Although an upper D class boundary of 1400mg/*l* was considered acceptable, it was decided to adjust this boundary downward to 1100mg/*l* (Table 2.17) in order to be more protective. This adjustment would put TDS toxicology classes for DWAF monitoring points H4H019Q01 and H3H011Q01 (i.e. Vink River at De Gorree and Kogmanskloof River respectively) firmly in an unsustainable E/F class, which is consistent with the reduced SASS scores obtained for sites on these rivers

TABLE 2.17
FINAL HEALTH CLASS ALLOCATIONS FOR TDS / EC BASED ON TOXICITY DATA. MODIFICATIONS ARE BASED ON SASS AND CHEMICAL DATA AND CATCHMENT CONDITION

Class	TDS (mg/*l*)	EC (mS/m)
A	< 45	< 7
B	45 – 300	7 – 45
C	300 – 800	45 – 120
D	800 – **1100**	120 – **170**
E	**> 1100** – 1700	**> 170** – 260
F	1700 – 2000	> 260

Total Dissolved Solids, or EC, is the first water quality variable considered due to its importance as a driving variable in river systems. TDS/EC are used as surrogates for salinity, and are particularly important in systems such as the Olifants River (Gauteng / Mpumalanga), where mining has resulted in increased salinization due to sulphate pollution, and in the Breede River, where the natural geology and irrigation return flows result in elevated NaCl levels. Bath et al. (1999) developed a preliminary method which was found unsuitable for linking TDS values to resource unit classes for the Olifants River, and the method incorporating toxicity results was developed (see Palmer and Rossouw (2001) and Palmer and Scherman (2000) for more information). Bath's method describes the reference condition for TDS in terms of the median, and classes for the PES are defined as deviations from the RC. When this method was applied to reaches in the upper Olifants River, most of the reaches were defined as E/F health classes with respect to TDS and EC. These classifications indicated a much poorer river health than the results from SASS invertebrate monitoring (Palmer and Rossouw, 2001). A toxicity approach was therefore developed. The TDS concentration-response relationship (DWAF, 2000a; Palmer and Scherman, 2000) was established and the experimental results provided a statistically valid estimation of the concentration of salts at which different percentages of the test population died. This toxicity data were then used in determining the PES for TDS (Table 2.15).

Case study

The case study presented in detail is that of the Breede River Basin Study. Figure 2.5 is a map of the study area, showing DWAF gauging weirs from where chemical data was collected. Water quality data collected described present state conditions (Rossouw and Kamish, 2000). SASS and habitat assessment data was available for each IFR site, and a spread of other sites throughout the catchment from Dallas et al. (1998) and the Breede River Basin Study (*Preliminary Assessment of the Breede River Basin, April 2000*). The level of confidence in the data is high. An unimpacted reference site was selected in each of the ecoregions, i.e. Cape Fold Mountains and the Cape Coastal Belt, and site-specific macroinvertebrates collected for toxicity testing (see Methods). Once toxicity data had been produced (Table 2.14), a table of health classes, based on TDS/EC toxicity data, was generated (Table 2.15) according to the descriptors in Table 2.13. These classes, together with the SASS classes, were depicted on a map of the study area (Fig. 2.5). The data (SASS and toxicology) were checked and evaluated (Table 2.16).

After consideration of all available data, it was decided to only adjust the boundaries of the D and E classes (Table 2.17), based on SASS (particularly ASPT; Average Score per Taxon) scores and associated TDS levels in the catchment, to be more protective. This example demonstrates the usefulness of assigning classes based on toxicity data and utilizing chemical and biomonitoring data to refine classes.

Discussion

Information on water physico-chemistry provides only limited information about river health. Frequently the information can only be interpreted in the context of biomonitoring and toxicity data which then provides results to set guidelines to protect rivers (Norris and Thoms, 1999). However, there are assumptions in the use of toxicological and biomonitoring data in the method presented in this section. An assumption of the toxicity test method used is that if the species used in the toxicity test do not show a toxic response then other species will also be protected. An important consideration therefore is the limitation of the toxicological data. However, this limitation can be addressed through further and more intensive testing using more species and other toxicants and the use of the more protective approach of the short-term chronic LC1.

A further assumption is that if biomonitoring data indicate that organisms (families) are present then they are able to withstand the variability experienced in water quality. However, there is no indication of which species may be absent, or why, as the biomonitoring data used (i.e. SASS data) only incorporates family level identification.

In addition to these assumptions, there are further limitations to the approach concerning the availability of data. Toxicity data are not always available, either due to a lack of experimental organisms or the impracticality of undertaking tests (e.g. expense or lack of other resources). For example, in the Olifants River study toxicology results were extrapolated for the length of the river as results of tests on organisms from the lower reaches were invalid due to high control mortality and time and budget precluded retesting. The results that were obtained seemed to be in the range of tolerances of the taxon tested in the upper reaches (Table 2.12). It would be more valid to repeat experiments using organisms from reaches in the lower and middle catchment, due to natural changes in water chemistry and invertebrate communities. SASS data are not always available where there are water quality monitoring points, or for all water quality resource units. Information may only be qualitative, and in addition, water quality data may also not be readily available, or limited variables may be monitored.

The need for research linking biomonitoring data to species concentration-response relationships has been stressed (Carlisle and Clements, 1999) and has further been highlighted by the studies presented here. It is important to understand the relationships between ecological structure and function, biomonitoring data and water quality data for sustainable management of aquatic resources.

The approach detailed in this section seeks to make the best use of available data and methods for effectively and efficiently assessing the quality Reserve or the water quality component of an environmental flow assessment. However, the application of the toxicity testing approach is under development and while appropriate to a system driven by salinization, such as the Olifants and Breede Rivers, it is of less use in a catchment driven by nutrient enrichment, such as the Thukela (although still necessary). The application of these tools, particularly toxicology, is therefore catchment specific and still under development for driving variables other than salinity.

An understanding of individual aquatic ecosystems function is central to the effective management of these ecosystems (Hart et al., 1999). In ecological Reserve assessments detailed ecological studies are not usually possible (insufficient resources, time and budget) yet specialists are required to make judgements on the ecological functioning of these systems to ensure their long-term ecological sustainability. In order to do this, best practice would be to utilise all data and information which is available or can be generated within the confines of the study. In water quality Reserve assessments, water chemistry data are readily available (from DWAF) and biomonitoring data are sometimes available. Site-specific toxicological data can be generated within two weeks of test taxon collection and provides a valuable link between water quality data and biomonitoring data. It would therefore be optimal to utilise the information in a pragmatic approach. The method presented here describes such an approach, and provides the starting point for further development of methods and models to incorporate water quality in ecological Reserve assessments.

2.2.5 An alternative approach

In relating salinity tolerance test results to ecological and management classes, Jooste (pers. comm.) is of the opinion that salts, rather than ions, TDS or EC should be the basis for regulation. While he recognises that there are important biotic effects related to high composite salt concentrations, he argues that there is strong evidence that the effects of individual salts will outweigh the effects attributable to TDS. He has recently interrogated the international database for the salt tolerances of a wide range of freshwater organisms and related the data to ecological health and management classes (Table 2.18).

The boundary values between classes were derived from the tolerance data of a wide range if freshwater taxa (Table 2.19). The approach was based on that of Aldenberg and Slob (1993), where the distribution of species tolerances is assessed, and values derived which are protective of a specified percentage of species for either acute (short term) or chronic (longer term) exposures. The acute exposure measure is mortality, the chronic exposure measure is some form of sub-lethal measure.

The Natural boundary values were based on international chronic effect data similar to the CEV (Chronic Effects Value) data (DWAF, 1996), extrapolated to protect 100% of species. The Fair boundary values were based on data similar to AEV (Acute Effects Value) data (DWAF, 1996). In a Fair class, 100% of species would not be acutely affected. The Good boundary value is linearly interpolated.

Jooste's values were derived for use as generic, first-level estimates of the relationship between salt tolerances and resource health classes, with the idea that they would be refined by site-specific studies.

However there is a fundamental difference in approach. Jooste (pers.comm) and Aldenberg and Slob (1993) used acute and chronic data from the widest possible range of taxa, and used LC50 values. In this study we have used one or two species from a single river or river reach, and therefore used LC1 and LC5 data, and the 95% confidence limits around these values (Confidence limit values have been termed "inferred" end-points (Tables 2.3 and 2.4)).

There is still a need to critically evaluate the two approaches and decide on their appropriate use.

TABLE 2.18
RIVER HEALTH AND INTEGRITY CLASSES IN RELATION TO ECOLOGICAL AND MANAGEMENT PERSPECTIVES (UYS ET AL., 1996; HOHLS, 1996; ROUX, PERS. COMM.) (THIS TABLE IS REPEATED, AND REFERRED TO IN CHAPTER 4)

Class	Ecological perspective	Management perspective
Natural A	Minimal or negligible modification of in-stream and riparian habitats and biota. The best Natural rivers are in the Reference or unmodified condition.	Protected rivers; relatively untouched by humans; no discharges or impoundments.
Good B - B/C	Ecosystems essentially in good state; biodiversity largely intact.	Some human-related disturbance but mostly of low impact potential.
Fair C/D - D	A few sensitive species may be lost; lower abundances of biological populations are likely to occur, or sometimes, higher abundances of tolerant or opportunistic species occur.	Multiple disturbances associated with need for socio-economic development, e.g. impoundment, habitat modification and water quality degradation
Poor E	Habitat diversity and availability have declined; mostly only tolerant species present; species present are often diseased; population dynamics have been disrupted (e.g. biota can no longer breed or alien species have invaded the ecosystem).	Often characterised by high human densities or extensive resource exploitation. Management intervention is needed to improve river health (e.g. to restore flow patterns, river habitats or water quality).

TABLE 2.19
CLASS BOUNDARIES FOR INORGANIC SALTS

Variables	Natural boundary (mg/l)	Good boundary (mg/l)	Fair boundary (mg/l)
Na_2SO_4	12	48	84
$MgCl_2$	9	37	64
$CaCl_2$	17	85	152
$MgSO_4$	59	165	271
$CaSO_4$	21	495	968
NaCl	3	602	1200

2.2.6 Conclusion

The are many challenges ahead. As a South African database is developed the Aldenberg and Slob (1993) method can be applied to SA regional data sets and the results compared with those for the whole international database. The role of site-specific work also requires critical evaluation. Methods for relating other water quality variables to resource health classes need to be developed.

The results of this study follow on from previous work, and new projects on environmental water quality requirement methods. Applied aquatic toxicology will build on these results.

CHAPTER 3

WHOLE EFFLUENT TOXICITY (WET) TESTING

3.1 REVIEW AND APPLICATION OF WET RESULTS

Water quality is defined by its physical, chemical, biological and aesthetic characteristics and determines the health and integrity of aquatic resources (DWAF, 1996). Management of water quality in aquatic resources have been implemented world-wide, mainly in response to deteriorating conditions, and various monitoring tools such as chemical monitoring, biological monitoring and toxicological assessments have been implemented. These tools are supported by on-going research and development in an attempt to improve strategies and approaches to water quality management.

In South Africa, the National Water Act (NWA) (No 36 of 1998) gives aquatic ecosystems the right to water quality which will ensure long-term sustainable use of the resource. In order to achieve this, the NWA provides for the implementation of *resource directed measures* (where aquatic ecosystem requirements are provided through quantitative and qualitative resource quality objectives) and *source directed controls* (which controls the quality of the sources of impacts, i.e. pollutants, through a licensing system). However, the balance between water quality for a healthy functioning ecosystem while still allowing use of the resource is difficult to achieve and can be partly addressed through better knowledge and understanding of the effects of pollutants (toxins) in aquatic ecosystems.

Water quality guidelines, which are single value limits for individual water quality constituents, are commonly used to manage and protect aquatic resources (Warne, 1998). However, this approach is limiting as there are usually no provisions for allowable variability of the guideline values (to allow for seasonal and flow variability) and is therefore not particularly effective for protection and management of aquatic resources (Hart et al., 1999). In addition to the limitations of water quality guidelines, most pollutants, defined as dissolved and suspended substances which adversely affect people and/ore the natural environment (Rand, 1995), enter aquatic resources as complex mixtures. These complex mixtures, or discharges, need to be identified and controlled for effective water quality management. However, discharges mix with water in aquatic resources and new chemical mixtures can be formed. Use of water quality guidelines, i.e. substance-specific limits, are of limited use in controlling or managing the impacts of the complex discharges in the environment. There are several reasons for this:

- mixtures may contain substances which cannot be individually identified;
- there may be too many chemicals, or they may be too expensive and/or too difficult to analyse;
- some substances may be present below chemical detection level yet still result in adverse environmental effects;
- biological processes may result in the formation of new mixtures which may be difficult, or impossible, to identify; and
- effects of individual substances may have different environmental effects than the combined effects of complex mixtures.

Yet, in order to manage, and protect, aquatic resources, it is vital to be able to quantify the effects of pollutants on those resources (Sarakinos and Rasmussen, 1998). Whole Effluent Toxicity (WET) testing, or Direct Toxicity Assessment (DTA), is considered suitable for being able to predict in-stream environmental effects (Ausley, 2000; Chapman, 2000) as one of the underlying assumptions of WET tests is that laboratory toxicity tests are predictive of effects in receiving water resources (Dorn, 1996). In WET testing, a range of test organisms is exposed to various

concentrations of a complex discharge and the effect on organisms is quantified (Grothe et al., 1996). These results are then used to set license specifications for effluent discharge in terms of toxicity test end-point rather than chemical concentrations. WET testing can be used to i.) establish the suitability of a discharge; ii.) develop permit limits to control effluent discharge; iii.) monitor effluent discharge; iv.) identify and prioritize effluent and v.) reduce toxicity of effluent (Slabbert et al., 1998a).

Effluent toxicity testing can be used effectively as a regulatory tool to manage the quality of receiving water (Stewart, 1996) and inclusion of whole effluent toxicity tests in effluent permits can ensure that effluents do not adversely affect aquatic life (Garric et al., 1993; Grothe et al., 1996). In South Africa, the Department of Water Affairs and Forestry (DWAF) has recognized the use of WET testing as a tool for assessing effluents for discharge to receiving waters (Slabbert et al., 1998a). However, there are a number of issues which need to be addressed in order to ensure successful implementation of regulatory toxicity tests for effluent discharge licenses and these relate to the methods and approaches of WET testing.

WET testing assumes that laboratory test results are accurate predictors of environmental effects of discharges and that, even though there may be some variability, WET tests are reproducible (Ruffier, 1996). However, there are a number of conditions which affect WET test variability. WET is relative to the test organism used, lifestages (or ages) tested, test method, the effluent itself, test conditions and the benchmark used to gauge sensitivity (Warren-Hicks et al., 2000). These factors will affect the outcome of the toxicity assessment (i.e. introduce variability) of an effluent and it is therefore important to control, or reduce, as much of the variability as possible. There are several WET test methods currently employed by the United States Environmental Protection Agency (US EPA) and other regulatory authorities following standardised procedures and standard laboratory organisms (Chapman et al., 1996). However, this does not preclude the refinement of these tests, nor the development of new methods, especially methods for additional test organisms, which will allow more accurate predictions of environmental effects.

The approach to evaluation of an effluent depends on the selected WET test method and conditions of the test (e.g. exposure system, dilution water, concentration range and experimental design), the test organism/s and the chemicals present in the effluent (Warren-Hicks and Parkhurst, 1996). These choices may further be influenced by whether the toxicity test to be undertaken is an acute or chronic toxicity test. Regulatory WET testing is usually carried out on limited test species, following restricted toxicity test methods, even though these species may not necessarily be the most sensitive species (sensitivity is influenced by species and toxicant being tested): standard test methods tend to use surrogate, or indicator, species which may not necessarily be in the water resources in question. Selection of test organism (and therefore the exposure system) is guided by several considerations, such as known sensitivity to a range of chemicals; ability to show rapid responses which are easy to quantify; abundant year-long supply of organisms which are easy to handle and are able to withstand laboratory conditions (Rand, 1995).

There are two different approaches to choice of test organism. The first is to use standard laboratory test organisms which have been reared in the laboratory under stringent conditions, while the other is to use wild-caught organisms representative of the study area (Chapman, 1995). Use of indigenous test species in toxicity tests has been widely recommended (Cairns, 1993; Chapman, 1995) to increase environmental realism. However, in order to make accurate

predictions of environmental effects it is necessary to make test methods (and choice of test organism and experimental test end-point) as environmentally realistic as possible. Obtaining environmentally realistic results remains challenging and part of the challenge is to select exposure (test) systems which are appropriate for test organisms: for example, it is not appropriate to expose lotic organisms to test solutions in a static test system.

The lack of toxicity data for lotic organisms may be a reflection of the lack of experimental test methods to generate the necessary data (Williams et al., 1984). Development of artificial stream systems world-wide has allowed for the generation of data for riffle-dwelling invertebrates in order to predict effects on lotic environs (Guckert, 1993; Palmer et al., 1996; DWAF, 2000; Palmer and Scherman, 2000). Use of artificial streams incorporates a measure of stream (or natural) variability while still maintaining a high level of experimental control (Canivet and Gibert, 2002). This allows for a better approximation of the potential effects of effluents in the natural (flowing water) environment. For this reason, the use of the newly developed standard method (DWAF, 2000) was assessed with regard to its use in assessing effluent discharges.

3.1.1 Application of the DWAF (2000) protocol for use in WET tests

In South Africa, rivers are important aquatic resources and are used extensively to supply water as well as dilute, transport and process waste and effluent. Yet there are no commonly-used test organisms from flowing water and nor has there been an attempt to obtain tolerance data for indigenous organisms. The protocol developed at the Centre for Aquatic Toxicology, Institute for Water Research (Palmer et al., 1996; DWAF, 2000; Palmer and Scherman, 2000) focussed on use of indigenous riverine invertebrates in artificial streams in order to obtain toxicological data relevant for South African environmental conditions. The method was developed using single substances (reference toxicants), but it was also necessary to investigate the application of the standard method for assessment of effluent discharge.

It is widely acknowledged that the acceptability of a toxicity test method is important in establishing its usefulness: standard toxicity test methods need to be reliable, precise and accurate and should have appropriate biological indicators (i.e. organisms and test end-points) (Robinson, 1989). Assessment of whether an acute toxicity test method is acceptable as a standard method is an assessment of whether toxicity test results can be replicated and statistical measures of certainty can be ascertained (Kimball and Levin, 1985). In order for a method to be accepted as a standard method requires that well-established test protocols be available and that these methods be acceptable to the scientific community (Rand, 1995). Repeated testing of a newly developed method will allow the establishment of a database of toxicological data and method-use information which will allow assessment of suitability and appropriateness of the said method. Interrogation of data can then provide a statistical assessment of the validity (repeatability and accuracy) of the new method.

In order to assess the suitability of the standard protocol using riverine invertebrates, two separate studies were undertaken in which wild-caught invertebrates were exposed to effluent in channel artificial stream systems in a series of experiments (Zokufa, 2001; Muller and Palmer, 2002). In each of the studies a series of acute 96-hour toxicity tests were undertaken to assess the toxicity of effluents. A summary of the experimental details is presented in Table 3.1. Artificial effluent was constructed for one of the studies (Muller and Palmer, 2002) for two reasons. Despite having access to a large array of effluents in the study area during the study period, it proved impossible

to find appropriately acutely toxic effluents in the Vaal Triangle. As a result, an artificial effluent was constructed. The advantage of this was that the test effluent remained the same over the study period and data could then be analysed to assess repeatability of the toxicity tests, which aided assessment of the protocol for use in WET tests. A further difference between the studies by Muller and Palmer (2002) and Zokufa (2001) was a modification of the channel artificial streams: in the study by Muller and Palmer, streams were modified to a "closed system", i.e. the outfall water from the stream and the water in the sump (bucket) was closed to minimize loss of water from the stream system.

TABLE 3.1
SUMMARY OF EXPERIMENTAL DETAILS USED TO TEST THE NEWLY STANDARD METHOD FOR USE IN EFFLUENT ASSESSMENT

Effluent type	River (Province)	Organisms tested	Stream design	Diluent	Reference
Kraft Mill Effluent	Sabie River (Mpumalanga)	*Tricorythus tinctus* (Tricorythidae)	open system	Sabie River water	Zokufa (2001)
Textile Effluent	Buffalo River (Eastern Cape)	Baetidae*	open system	Buffalo River water	Zokufa (2001)
Artificial Effluent	Vaal River (Gauteng)	*Afronurus peringueyi* (Heptageniidae) *Euthraulus elegans* (Leptophlebiidae)	closed system	Vaal River water	Muller and Palmer (2002)

*Species complex

All experiments were undertaken on-site (i.e. near organism collecting sites) and, in order to increase environmental realism, river water from an unimpacted site was used as diluent for each of the site-specific studies. Wild-caught indigenous organisms were collected from reference (unimpacted) sites and sorted into artificial streams upon arrival in the laboratory; a minimum of 25 organisms was placed into each of the channels. Organisms were allowed to acclimate to laboratory and stream conditions for 36 hours before they were exposed to effluent. Any mortalities which occurred during the acclimation period were removed and duly recorded. Acclimation mortality was always less than 10%, as per recommendation (DWAF, 2000). A range of effluent concentrations was selected from 100% effluent to 0% effluent (control) and organisms were exposed to the effluent in a regression experimental design.

As acute toxicity tests were undertaken, the test end-point selected was mortality. This is an easily definable and, usually, unambiguous end-point which is commonly used in acute toxicity tests (Chapman et al., 1996). Exposure systems were checked twice daily for mortalities: dead organisms were removed and preserved in 80% alcohol. After 96 hours, all remaining live organisms were collected and preserved in 80% alcohol. The data were used to calculate point estimates using Probit or Trimmed Spearman-Karber analysis techniques (see Appendix 3 and 4 for more detail). Data from the two studies were used in different applications, but both studies can be used to evaluate the application of the standard protocol for use in WET testing.

Zokufa (2001) represented the first step in extending the protocol for indigenous riverine organisms for use in WET testing. Findings of the study (Appendix 3) indicated that both kraft and textile effluent were highly variable and most samples were acutely toxic to the selected test organisms (*Tricorythus tinctus* and Baetidae, respectively). Point estimate data (LC1, LC5, AEV and CEV) were used to generate hazard-based percentage guidelines for allowable percentage effluent discharges to the receiving systems to allow for different levels of protection of the aquatic resource. Further testing of the effluents, with a more comprehensive list of test organisms, can be used to refine these guidelines, and possibly even allow for seasonal variability.

Results from the study by Muller and Palmer (2002) indicated that there were significant differences between organism responses to the same effluent with *Euthraulus elegans* being significantly more sensitive than *Afronurus peringueyi*. As a result, confidence in use of the selected test organisms for routine, or regulatory, monitoring, differed (Appendix 4): greatest confidence could be placed in *E. elegans* as the % Coefficient of Variation (%CV), which was used as a measure of precision of the WET test results, was lowest for this organism (it was also lower than the %CV for *Daphnia pulex*, a standard laboratory test organism). Of interest is that results suggest that use of indigenous invertebrates and the test method are appropriate for use in WET testing.

The relative toxicities of the different effluents could not be ascertained as different test organisms were used and benchmark test organisms (i.e. standard test organisms such as *Daphnia pulex*) were only used for one of the studies (Muller and Palmer, 2002). This is unfortunate as using benchmark species in initial toxicological assessments may be useful to highlight effluents for immediate and further investigation, i.e. prioritisation of effluents of concern.

Neither study allowed for the examination of seasonal impacts of variability of both test organisms and effluent variability.

3.2 Evaluation of the Application of the Protocol for WET testing

Acute WET tests provide a useful and important first screening step in assessing the toxicological effects of chemicals and effluents. It has been suggested that the systematic generation of toxicological data is a good approach to determine permissible limits of toxicants/effluents (Williams et al., 1984) and that site-specific application of these toxicity tests should be included (Chapman, 2000). Data presented in the studies confirmed that whole effluent toxicity tests using indigenous riverine invertebrates in artificial stream channels can be used to assess effluent discharge as organisms respond (increased mortality) to increased concentrations of effluent.

However, the presented studies also served to highlight issues and questions which contribute to variability in WET test and may result in confusion in the application of WET test results in water quality management. It should be stressed that these issues apply to toxicity tests in general and not necessarily only to the standard protocol described in this chapter; furthermore, issues raised are also pertinent to single-substance and reference toxicant toxicity testing. It is only through further application of the standard protocol using both single substances and whole effluent that an attempt can be made to understand these issues.

Recent reviews of toxicity test variability, with particular reference to WET test variability, in

an attempt to define allowable variability (especially for regulatory purposes), have proved to be insightful. The impact of intra- and inter-laboratory variability was examined using standard toxicity tests and reference toxicants. Moore et al. (2000) selected data from four laboratories, using two species and two reference toxicants. After initial screening of the available data, they selected between 9 and 56 exposure tests to take forward in their analysis. Warren-Hicks et al. (2000) examined a dataset containing information on nine test species, nine reference toxicants, 11 test methods and 1788 reference toxicity tests. Many of these tests were eliminated from the final assessment and an important factor which resulted in inclusion in the analysis was the concentration ranges selected for the toxicity tests. The US EPA (2000) assessed data from 75 laboratories, covering 23 methods over an 11 year period, from 1988 to 1999. An important feature of these papers is that reference toxicity testing provides a substantial basis for the assessment of variability and the contribution that method variability and organism variability can make to effluent assessment. All tests examined used laboratory-reared cultures in the standard toxicity tests: it appears that no effort was made to include an investigation of use of wild-caught organisms in toxicity tests.

Although we have begun accumulating data for the standard protocol using wild-caught indigenous invertebrates in artificial streams, our database is extremely limited. We have few tests using reference toxicants (Chapter 2.1), using an array of species from different rivers. The studies presented here provide an initial and important contribution towards a toxicological database for indigenous invertebrates exposed to whole effluent. This in itself is a major contribution towards water quality management and use of a risk-based approach to setting integrated environmental objectives to protect aquatic resources. Unfortunately, detailed statistical analysis of use of the standard protocol for WET tests using indigenous organisms is not yet possible.

However, it is possible to make observations on the application of the standard protocol for WET testing using results from the WET studies and use these to make recommendations for future work. There are subtle aspects of the standard protocol which are not immediately apparent but which may introduce undesirable variability to the method.

- *Identification, and handling, of suitable organisms for the toxicity test from a reference (or least impacted) site.*

Standard laboratory organisms are reasonably easy to culture thereby ensuring a supply of test organisms. Using wild-caught organisms adds a level of uncertainty: it is not clear which species will be available at which time of year in sufficient numbers. This is not always possible to predict, as the biology of most indigenous invertebrates remains unresearched, and selection of test organisms is therefore left to the discretion of the toxicologist undertaking the test. This is inherent in the method, and not only associated with the application of the method for WET testing. However, until significantly more testing has been done, it is not possible to assess the influence of species on the method.

- *Experimental design, choice of concentration range and dilution medium.*

These factors are known to influence the experimental end-point (Muller and Palmer, 2002). When the standard protocol is used as initial screening test discretionary choices for experimental design, concentration range and dilution medium may be allowable. However, if the method is

to be applied in a regulatory context, it is recommended that fewer choices be afforded to minimize variability in results, as precision of test results is of concern (Moore et al., 2000). The circumstances and conditions for choices, and the expected outcomes, need to be defined clearly in the protocol.

In the USA, the preferred statistical method in whole effluent toxicity assessments for the NPDES Permit Program is to obtain point estimates as test end-points (Lewis et al., 1994), usually best obtained through a regression experimental design. However, if the preferred experimental design is a replicated approach, the recommendation is to use a minimum of 4 replicates (Lewis et al., 1994). Using a replicated experimental design, it is possible to establish with greater confidence the amount of organism variability. However, this organism variability also obscures test precision and result in less confidence in the LOEC (lowest observed effect concentration) and subsequent calculated NOEC (no observed effect concentration) (Gully et al., 1999). This may have implications for laboratories undertaking the toxicity tests as test systems (i.e. individual channels) require more space than test vessels for other standard tests. The choice of experimental design may be influenced by available space. This in turn will impact on the concentrations selected for the exposure test.

The selected concentration-range for the exposure experiment showed the greatest source of variance in an analysis of standard methods and organisms using reference toxicants (Warren-Hicks et al., 2000). For this reason, it may be necessary to define (specify) concentrations to be used when testing effluent; the concentrations may be effluent-specific, but this can only be determined through further exhaustive testing.

The current standard protocol is for a 96-hour procedure (DWAF, 2000). While this has successfully been extended to a 10-day exposure period (sub-lethal testing) for single-substance testing, similar tests using effluent have not been undertaken with any great degree of confidence. There are several issues which arise, pertaining mostly to effluent and its storage.

- *Effluent type, grab or composite samples, and storage of effluent*

Only a limited number of effluents have been tested in the channel artificial streams. Some attempt was made to characterise changes in effluent over this exposure period (Zokufa, 2001), but no particularly volatile effluent has been tested. In order for the standard protocol to be used for effluent toxicity assessment, it will be necessary to include guidelines in the protocol for the use and handling of effluent; at this stage, it is recommended that guidelines set up by the US EPA be followed.

Further use of the standard protocol (testing a wider range of effluents) may highlight issues not addressed here and it may then become necessary to make adjustments to the method. However, it has been recommended that any changes in a standard protocol be *rigorously* examined experimentally in order to provide supporting evidence necessitating the change (Markle et al., 2000). Where it is possible to deviate from the standard protocol, and evidence of consequences of these deviations are unknown, it must be made explicit in the standard method what the potential consequences are so that toxicologists can make informed decisions.

3.2.1 Recommendations for future work and research

There were several issues highlighted by the WET testing studies undertaken which lead to further research requirements.

i.) Technology transfer and training of competent technicians is vital.

A major component of variability has been found to be attributable to choice of laboratory (Warren-Hicks et al., 2000). Unfortunately the protocol has been tested by few people besides those who were involved in its development and standardisation. Until now, only CAT-IWR (or associated students) has used the method to generate toxicological data. This in itself presents a problem. Technology transfer and training of personnel is urgently required in order to develop an adequate data set so that a comprehensive assessment of the method can be undertaken.

ii.) The use of a positive control testing, and reference toxicity testing, when undertaking effluent toxicity tests should be examined in detail: this can be used to test the relative responses of test organisms over time and from different populations.

This may be used to assess whether changes in toxicity test results may be due to changes in the effluent or changes in sensitivity of test organisms.

Testing of reference chemicals such as sodium chloride and sodium sulphate allowed the development of a standard protocol for single substances using wild-caught indigenous macroinvertebrates in artificial streams (DWAF, 2000). While this method was tested for some reference toxicants, it has not yet been benchmarked using other reference toxicants such as cadmium, mostly due to practicalities, such as disposing of large quantities of highly toxic waste. However, it has become obvious that there may now be a need for this kind of further testing.

iii.) If WET tests using indigenous invertebrates in artificial streams should become a regulatory requirement, it will be necessary to establish the frequency of testing.

Organisms may show varying sensitivities to toxicants during different phases of their life-cycles. As a result, it may be necessary to find optimum testing times for wild-caught populations.

iv.) Development of indigenous invertebrates as standard laboratory-reared test organisms for use in aquatic toxicity tests.

Commercial laboratories undertaking routine toxicity tests are unlikely to collect wild-caught indigenous invertebrates for use in aquatic toxicity tests, despite evidence showing the value of using these organisms (du Preez, pers. comm.). Use of laboratory-reared indigenous organisms in aquatic toxicity tests presents a number of advantages, most notably a constant supply of test organisms as well as known prior exposure conditions. However, laboratory rearing methods are not readily available for indigenous riverine invertebrates. At present, CAT-IWR is refining methods for breeding and use of the freshwater shrimp, *Caridina nilotica*, as a laboratory-reared indigenous organism. It is therefore important to continue on-going investigations into the development of standard methods for maintaining laboratory cultures of indigenous invertebrates, as well as appropriate toxicity test methods for these organisms.

CHAPTER 4

CONTRIBUTIONS TO POLICY DEVELOPMENT AND IMPLEMENTATION

4.1 INTRODUCTION

There have been two main contributions to policy development and implementation:

- The development of methods for assessing water quality within ecological Reserve assessments (Palmer et al., 2000; Palmer and Rossouw, 2001; Palmer et al., in prep.; Scherman et al., in prep.; Chapter 4.2).
- The development of a discussion document and supporting technical guidelines for the implementation of toxicity testing in the source directed control of complex waste discharge and impact (DWAF, in prep; Chapter 4.3)

4.2 THE DEVELOPMENT OF METHODS TO INTEGRATE WATER QUALITY INTO RIVERINE ECOLOGICAL RESERVE DETERMINATIONS

The content of this section has been submitted for publication to *Rivers Research and Application.*

4.2.1 Introduction

The ecological Reserve

The two founding principles of the South African National Water Act (No. 36 of 1998) (NWA) are "sustainability" and "equity" (NWA, 1(1)(xviii)(b)). These principles are supported by acknowledging that the water cycle is an integrated process and should be managed as such. Particular attention should be paid to integration between water quality and quantity; ground and surface water; and between rivers, impoundments, wetlands and estuaries. In the NWA there are only two rights to water: water for drinking, cooking and hygiene, and water for sustainable ecosystems. All other water (for industry, agriculture, domestic use and waste disposal) is allocated by licence. The two water rights are provided for by the *Reserve*, comprising the basic human needs Reserve and the ecological Reserve, and this paper deals only with the latter.

Goods, services and classification

In any developing country, the optimal use of natural resources for healthy economic activity is essential. It is therefore necessary to provide people with choices, and information about the goods and services offered by aquatic ecosystems (Palmer et al., in press). A river classification system aims to optimise sustainable resource use, by providing an organised basis for identifying and choosing river condition and for setting descriptive and quantified resource quality objectives. The choice of river health can be linked to the goods and services offered by rivers, which include: water supply; waste transport, processing and dilution; natural products (e.g. reeds, fish, medicinal plants); nature and biodiversity conservation; flood control; recreation; aesthetic needs; and sites for religious rituals or spiritual needs. Aquatic ecosystems can, if managed appropriately, continue to supply people with these goods and services into the future, and therefore implementation of the NWA involves "resource protection to ensure sustainable resource use" (DWAF, 1997).

However, aquatic ecosystems cannot offer the whole range of goods and services at the same time in the same place. For example, if heavy use is made of water supply and waste disposal –

then the ecosystem is unlikely to provide well for nature conservation, recreation or "a sense of place". Therefore people need to be able to choose which services they want from which ecosystems in time and space. The degree of "naturalness" of an ecosystem is equated with ecosystem integrity and health (Hohls, 1996; Uys et al., 1996). The underlying assumption is that completely natural systems have the highest possible level of ecosystem integrity and health in terms of both structure (habitat and species composition) and function (processes such as carbon breakdown, photosynthesis and respiration) (Allan, 1995). As ecosystems are used, their structure and functioning changes. Most of these changes result in a deterioration of ecosystem integrity and health. In order to enable choices about the kinds and degrees of aquatic ecosystem use, and therefore choices about naturalness and ecosystem health and integrity, a river health classification system has been developed in South Africa.

A variety of classification approaches have been reviewed (Uys, 1994). The selected classification system ranks ecosystem health along a continuum, or gradient, from natural to poor (or even very poor). For effective resource management, "fuzzy" boundaries are imposed along this gradient, and descriptions of the central characteristics for four classes/categories provided: Natural, Good, Fair and Poor. Each of the classes is associated with a level of ecosystem health and integrity (Table 4.1), and the potential to offer a particular range of goods and services (Palmer et al., in press). For water quality, each class is quantified by boundary values with adjacent classes. Where data do not allow quantified ranges, qualified descriptors may be included.

TABLE 4.1

RIVER HEALTH AND INTEGRITY CLASSES IN THE RELATION TO ECOLOGICAL AND MANAGEMENT PERSPECTIVES (UYS ET AL., 1996; HOHLS, 1996; STATE OF RIVERS REPORT, 2001; ROUX, PERS. COMM.)

Class	Ecological perspective	Management perspective
Natural	Minimal or negligible modification of in-stream and riparian habitats and biota. The best Natural rivers are in the Reference or unmodified condition.	Protected rivers; relatively untouched by humans; no discharges or impoundments.
Good	Ecosystems essentially in good state; biodiversity largely intact.	Some human-related disturbance but mostly of low impact potential.
Fair	A few sensitive species may be lost; lower abundances of biological populations are likely to occur, or sometimes, higher abundances of tolerant or opportunistic species occur.	Multiple disturbances associated with need for socio-economic development, e.g. impoundment, habitat modification and water quality degradation
Poor	Habitat diversity and availability have declined; mostly only tolerant species present; species present are often diseased; population dynamics have been disrupted (e.g. biota can no longer breed or alien species have invaded the ecosystem).	Often characterised by high human densities or extensive resource exploitation. Management intervention is needed to improve river health (e.g. to restore flow patterns, river habitats or water quality).

Water quality in an ecological Reserve assessment

The task of an ecological Reserve assessment is to provide both quantified and descriptive information about the pattern and reliability of environmental flows, with information on flow frequency, magnitude and duration, so that an entire modified flow regime is provided (King et al., 2000). However methods for quantifying environmental water quality still focus on only magnitude (concentration), and frequency and duration are only taken into account via flow-concentration modelling (Malan and Day, in prep.). During an ecological Reserve assessment, the ecological Reserve for water quality is provided as class boundary-value concentrations for each variable.

From the many possible water quality variables, the initial suite to be considered in South Africa includes: inorganic salts (sodium chloride, sodium sulphate, magnesium chloride, magnesium sulphate, calcium chloride, calcium sulphate); nutrients (phosphate as PO_4^{-3}, and total inorganic nitrogen); physical variables (turbidity, pH, oxygen, and temperature); and those toxic substances listed in the South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996). In addition, bioassessments such the aquatic invertebrate index SASS (Chutter, 1994; 1998), algal abundance (Barbour et al., 1999) and toxicity tests (Slabbert et al.,1998a and b; Palmer and Scherman, 2000; Palmer and Rossouw, 2001), will be undertaken (DWAF, 2001).

General

The products of an ecological Reserve assessment are quantified and descriptive information about flow and water quality requirements. These end-points are linked to the complexity of ecosystem structure and function through components that assess habitat (hydraulic and geomorphological), biota (fish, invertebrates, vegetation - riparian and instream), and the responses of biota to the stress of altered flow and chemical variables (O'Keeffe et al., in press).

Although environmental flows are commonly provided for in many countries (review, King and Tharme, 1994), the effects of reduced, or altered flows, in relation to stable or increasing waste discharge and non-point source pollution is seldom considered. The most common approach to environment water quality protection is through protective water quality guidelines (Hart et al., 1999). The new Australian water quality guidelines (ANZECC and ARMCANZ, 2000) do allow for the selection of levels of resource protection, but do not link these to environmental flows. The aim of this paper is to outline a preliminary approach by which environmental water quality requirements can be managed in conjunction with environmental flows to achieve sustainable water resource management.

4.2.2 Water quality procedure

The procedure outlined here, and detailed in the DWAF (2001) manual, for assessing water quality in ecological Reserve determinations for rivers, requires that each water quality constituent, is described under a prescribed set of headings (Table 4.2). The main steps are described below, and shown in Figure 4.1.

TABLE 4.2

AN ASSESSMENT OF WATER QUALITY IN ECOLOGICAL RESERVE ASSESSMENTS FOR *RIVERS REQUIRES THAT EACH WATER QUALITY CONSTITUENT IS DESCRIBED UNDER A* PRESCRIBED SET OF HEADINGS

Heading	Description
Rationale	This section describes the effect of the constituent on the aquatic ecosystem and provides some justification for its inclusion in a water quality reserve determination.
Conditions for using this variable	This section provides rules for including the constituent in the reserve determination. Most constituents are obligatory but others, such as temperature, may only be included under specific conditions.
Benchmarks	This section describes the default benchmark values and provides some justification for the selection of specific benchmark values. Note: "Benchmark" is used here to mean a recognisable end-point, or condition than can be commonly identified, for example the natural condition, a threshold of probable concern or the chronic effects value. The default benchmark will be a set of class descriptors provided in the RDM manual (DWAF, in prep.)
Reference conditions	This section provides a set of rules that can be used to describe or model the unmodified condition, and may be used to adjust the default "Natural" benchmark values to site specific conditions using observed data.
Present state classification	This section provides a set of rules that can be used to link the present water quality status for a constituent to a class (Natural, Good, Fair or Poor). It also links some of the response variables to some of the constituents and provides rules to assess whether the constituent class is a true reflection of the class for the water quality resource unit.
Ecological specifications	This section provides guidelines about how to specify the quantitative and qualitative ecological specifications for a specific water quality resource unit.
Display and presentation options	This section shows examples of how the ecological specifications can be displayed or presented.
Additional information	In this section, any additional information that is pertinent to the assessment of a constituent is listed.
References	Any references are listed.

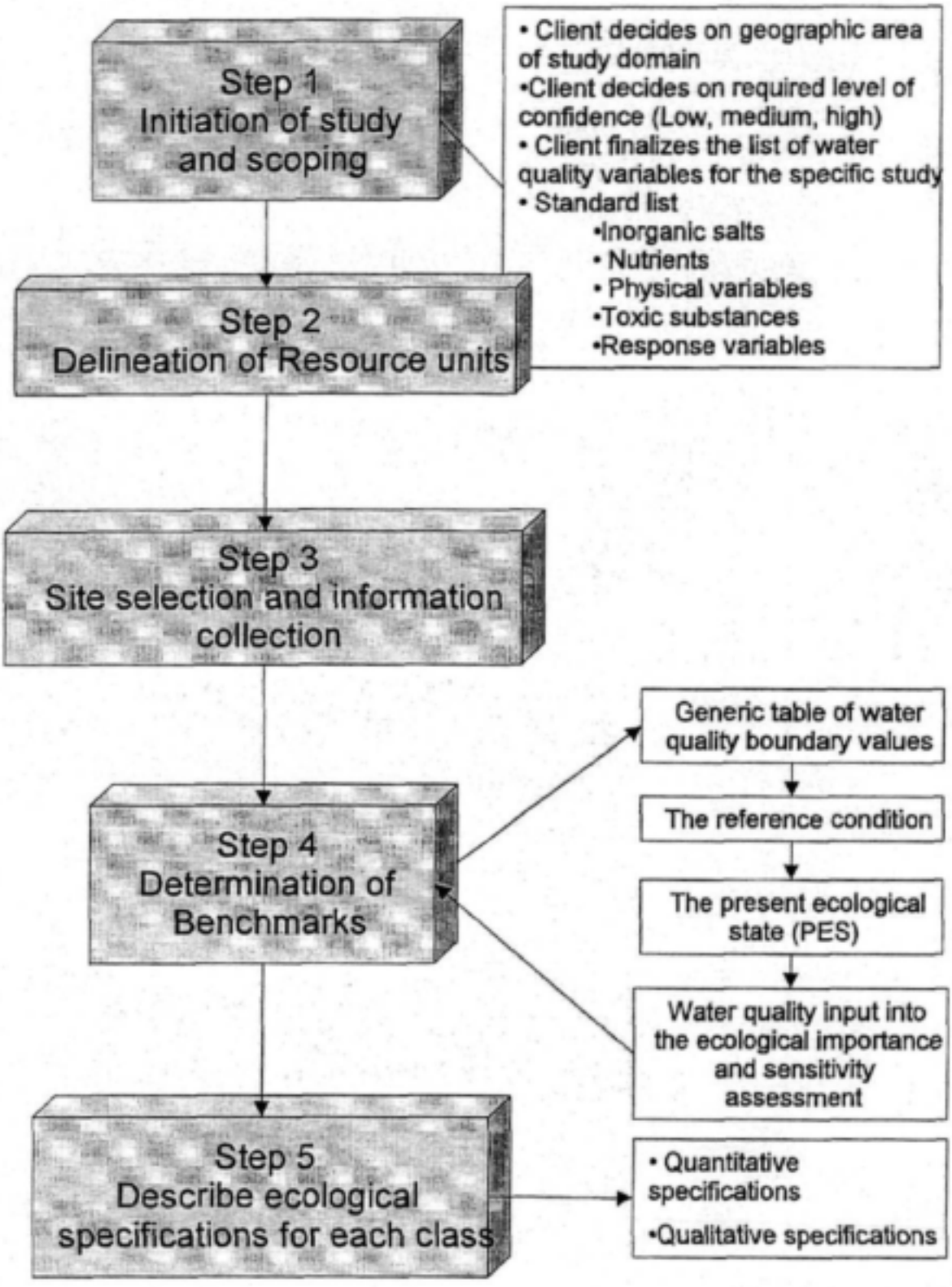

Figure 4.1
Roadmap of the five key steps for assessing water quality in an ecological Reserve determination for rivers.

Step 1 Initiate study and scoping

Study domain
This is the geographic scope of the study area, including the length of river, and the tributaries to be considered. It is important to include tributaries with water quality that is naturally or anthropogenically different from the main-stem of the river since poor water quality can cause "hot spots", and good water quality can provide biotic "refugia" and recolonisation sources (Palmer and Rossouw, 2001).

Level of confidence (high, medium, low)
The results of an ecological Reserve assessment can have differing levels of confidence, depending on the quality and extent of the available data. The quality of the data depends on the regularity and accuracy of monitoring programmes; the capacity to collect additional field and/or laboratory data specifically for the ecological Reserve assessment; and the availability of appropriate modeling tools. Depending on the constraints of the budget, available time and the quality of existing data, ecological Reserve assessments can be undertaken so as to produce high, medium or low confidence results.

Finalisation of water quality variables for the specific study
The standard suite of variables is usually be considered, but there may be additional specific variables of concern, for example, because of local geology, or because of discharges and impacts. Additional variables can be motivated on a site-specific basis.

Step 2 Delineation of resource units

A water quality resource unit is a length of river for which a single description of water quality can be given. This needs to apply both to natural, unimpacted streams and to impacted streams. For example, the natural water quality of the upper reaches of rivers is often different from the lower reaches, and therefore ecoregions (Kleynhans, 1999) are used to indicate resource unit boundaries. Additional resource unit boundaries are needed because certain impacts change the present water quality, and therefore dams, tributaries, towns and pollution point-sources need to be considered as appropriate resource unit boundaries.

Step 3 Site selection and information collection

Sites for data and information collection within resource units are identified and mapped, and each water quality resource unit is described by a set of water chemistry and bioassessment data. Where there are inadequate data either appropriate data from "equivalent" resource units are used or data are collected.

Step 4 Determination of benchmarks

Benchmarks comprise the key quantitative values, and qualitative narrative descriptions, that comprise the water quality component of an ecological Reserve assessment. There are five benchmarks:

- Generic Class/boundary value tables
  These are a set of generic tables, derived from the literature, which describe and/or quantify the boundary values between classes for each water quality variable. Where the tables are used without any corroboratory data (existing, or collected for the study) the ecological Reserve assessment will have a low confidence.

- Reference condition
  The reference, or natural, condition provides the benchmark against which to judge the class boundaries, and the present state, of each aspect of the water quality of the resource unit. The reference condition is the first benchmark to be adjusted on a site-specific basis after site-specific data are checked against the generic boundary value tables. The reference or natural condition is described using either pre-impact data, or using data from unimpacted sites. The Natural boundary equals the reference condition. In many resource units, and particularly in the lower reaches of rivers, there are no unimpacted sites, and reference conditions are difficult to infer. Data can be used from neighbouring catchments within the same ecoregion or an acceptable approximation of the natural condition.

- Present Ecological State (PES)
  The Present Ecological State (PES) is the measured, current water quality for each water quality resource unit and provides the point of departure for the development of any management objectives. Chemical and biotic response data are linked to a class (Natural, Good, Fair, or Poor). Only data from 1-3 years prior to the assessment of the PES are used. If the data record is poor (less than a monthly sampling frequency), then data from up to, but no longer than, 5 years prior to the assessment can be used.

If the results of the bioassessments are not consistent with the water chemistry (i.e. they indicate a different ecosystem health class), then there is a need for interpretation and adjustment of the concentration-class relationship. For example, if the presence of algal growth indicates a lower class than the nutrient chemistry, then nutrients should be described by the lower class; and/or in any resource unit where a SASS class that is lower than other the class indicated by water chemistry variables, an instream toxicity assessment is needed. If there is an indication of toxicity from response data, or from a chemical inventory, then a full toxicity assessment should be undertaken (DWAF, 2001).

- Ecological importance and
- Ecological sensitivity
  Water quality data are used in following the importance and sensitivity procedures of Kleynhans (1999), where the value of the natural water quality in relation to conservation status of the river, and its role in society, are assessed.

Step 5 Describe ecological specifications for each class

Quantitative specifications (Boundary values)
The quantitative ecological specification is a table that specifies: the water quality resource unit; the natural, good and fair boundary values for each variable examined in the assessment; the level of confidence (low, medium or high) associated with each variable; and any comments required

to clarify the boundary values that have been specified or the level of confidence for each variable.

Qualitative specifications
The qualitative ecological specifications is a narrative description that links the quantitative ecological specifications to site-specific information. During the assessment of the ecological water quality requirements, the water quality specialists gain insights about the water quality behaviour of the river system. (For example, the role of refugia and hot spots in tributaries or from point sources (Palmer and Rossouw, 2001).) These insights may not be captured in the quantitative ecological specifications, and this step provides the opportunity to document these insights in a concise manner.

4.2.3 Integration of water quality with environmental flow assessment

In South Africa, there are two accepted methods for ecological flow assessments for rivers, the Building Block Methodology (BBM) (King et al., 2000) and the Downstream Response to Imposed Flow Transformations (DRIFT) method (King et al., in press). The water quality assessment procedure fits into both methods.

The first step is to delineate the study area. After that, sites for environmental flow assessments, and water quality resource units are selected. The water quality resource units are river reaches which would naturally have a similar water quality, or currently have a similar water quality. Each unit which will be managed for a single set of water quality objectives (specifications that relate to a single ecosystem health class). Each water quality unit should include a data monitoring point, or at least an annual cycle of monitoring should be undertaken. Dams and tributaries may influence water quality, as could point-source discharges and large urban/industrial areas, and therefore may indicate unit boundaries.

Water quality data for each unit should be collected. Data should include values for water quality variables, bioassessment data, pollution data, toxicological data (Palmer and Scherman, 2000; DWAF, 2001; Palmer and Rossouw, 2001), provide toxicity-based methods for relating electrical conductivity, TDS, and individual salts to ecosystem health classes. Water quality data exploration can include scatter plots, trend assessments, plots of concentration against flow and time and box-and-whisker plots with summary statistics [5, 25, 75, 95 percentiles]). All monitoring points, land-use patterns and point sources should be mapped. Simple flow-concentration modeling can be undertaken (Malan and Day, in prep.). The results are used to write a water quality starter-document, for use in the ecological Reserve workshop. The BBM and DRIFT reports will include the relationship between flow and concentration, medium to long term trends in concentrations; the probable causes of water quality impacts; possible water quality management options, and the likely ease of implementation. The BBM requires quantitative and quality objectives for water quality, related to each ecosystem health class; and the DRIFT method requires a list of water quality variables and a systematic classification of how each variable can change in relation to flow changes (King et al., in press).

At the workshop, a group of specialists (in hydrological modeling, hydraulic modeling, geomorphology, riparian and in-stream habitat integrity, fish ecology, aquatic invertebrate ecology, riparian vegetation, social anthropology and water quality) collaborate to define the flow and water quality requirements for river resource units to function at different level of ecosystem

health. The water quality specialist provides information on the probable water quality consequences that result from particular flow reductions or increases. These consequences are reported in terms of chemical, biotic and toxicological responses to flow changes. In each resource unit, a modeled modified flow regime (Hughes, 2001) is provided for each ecosystem health class. An ecosystem health class that is attainable through management, and is consistent with the ecological sensitivity and importance of the unit, is recommended as the management goal for each resource unit.

4.2.4 Discussion

Pollution control – an essential management tool

Environmental flow is generally recommended at specific sites (extrapolated to river reaches) and takes account of riverine habitat integrity, geomorphology, and the needs of riparian vegetation, fish, and aquatic invertebrates (King et al., 2000; in press). Once an instream flow recommendation is agreed the water quality team considers the consequences to water quality of the recommended flows. If a situation arises where the *natural* water quality were to be impacted by recommended flows, then higher environmental flows would be recommended. (For example, if a stream had a naturally high salinity which would increase with lower flows to unacceptable levels). However, environmental flows are **not** recommended to address anthropogenic water quality impacts. This is important because the ecological flows are motivated to meet specific biological objectives, which would be obscured by adding flows to solve water quality problems.

However, in severely polluted rivers (Palmer and Rossouw, 2001) there is no doubt, that if only the recommended environmental flows remain in the river, without stringent application of waste discharge (DWAF, 1995) and non-point source pollution controls (Pegram and Gorgens, 2001), water quality will deteriorate still further. Pollution control is imperative if the resource is to be adequately protected. Where such controls may take time to implement, water resource managers should be alerted to river reaches that require intervention management, for example the use of dilution, to solve water quality problems that threaten resource health and integrity.

Refugia and "hot spots"

In most catchments there are reaches and tributaries with particularly good water quality, which play a particularly important role in the improvement of downstream water quality conditions, and potentially act as refugia for biota from adjacent, more impacted, reaches (Palmer and Rossouw, 2001). In the integrated management of the catchment the vital role of these good quality river reaches should not be under-estimated, nor the dependence of downstream river health on them overlooked.

Likewise, there are often reaches and tributaries that are severely impacted and have very low water quality; these negatively impact either on receiving impoundments, or on downstream reaches. These have been termed "hot spots" (Palmer and Rossouw, 2001). Effective source-directed controls in these reaches would significantly improve catchment water quality.

Risk and hazard

At present, the flow conditions that potentially allow an ecosystem to exist in each of the classes can be described in terms of the frequency, magnitude and duration (Hughes, 2001). However, for water quality variables, most class descriptions are in terms of magnitude (usually concentration) only, with duration and frequency only indirectly considered, if at all. Because of considering mainly magnitude, the present method is based on hazard descriptions, whereas future assessments may be risk-based (Jooste et al., 2000).

4.2.5 Conclusion

Compared with the assessment of environmental flows, the development of methods for environmental water quality assessments, and the integration of water quality with flow in protective water resource management, is in its infancy. Most of the elements of the procedure described here are currently being further researched, but we suggest that environmental water quality assessment and management is crucial for sustainable river management.

4.3 THE DEVELOPMENT OF GUIDELINES FOR THE ASSESSMENT OF COMPLEX WASTEWATER DISCHARGES

4.3.1 Introduction and background

(This section includes text by Sebastian Jooste, IWQS, DWAF)

This project has made direct input into formalising the use of toxicity test results in pollution control by DWAF. The project team have worked in conjunction with DWAF researchers working on the development of guidelines for the assessment of complex wastewater discharges.

The management of pollution over the past four decades has largely been based on setting and managing to levels of single substances in water. The success of this substance-specific approach in discharge management depends on knowledge of:

- the composition of the discharge, and
- knowledge of the effect of each component of the discharge.

In complex waste discharges either, or, both of these may be missing and therefore in itself the control of single substances may not be effective in assessing the environmental hazard of discharges containing mixtures of substances.

Where a **"complex wastewater discharge"** is defined as an " an industrial waste discharge which is a wastewater discharge containing more than 10% complex industrial wastewater, by volume, that is collected, treated, and subsequently disposed of.

AND

Where a " **complex industrial wastewater**" means wastewater arising from industrial activities and premises, that contains:

- a complex mixture of substances that are difficult or impractical to chemically characterise and quantify, or
- one or more substances, for which a Wastewater Limit Value has not been specified, and which may be harmful or potentially harmful to human health, or to the water resource

(identification of complex industrial wastewater will be provided by the Department of Water Affairs and Forestry upon written request).

General Authorisation, Schedule 3.6 (ii) and (vi).

There is a wide international acceptance of using chemical, substance-specific, limits in the control of environmental pollution. In South Africa this approach is exemplified by the general and special limit values. However, the substance-specific approach is of limited use in controlling the effects of discharging complex chemical mixtures which are commonly the result of industrial processes. In addition, industrial discharges often reach surface water resource via sewers and through sewage treatment works. The industrial discharge is therefore mixed with domestic sewage. In such complex chemical mixtures there are many substances that cannot be individually identified, and the number of substances can be too large, too expensive, and/or too difficult, to analyse. The analytical detection limit of some substances bears no correlation to the environmental effect. Partial degradation through biological treatment processes can produce mixtures that are either difficult or impossible to characterise. Mixtures can have substantially different environmental effects than the sum of individual substance effects, and frequently the environmental effects of even the identifiable substances are incomplete or unknown. Chemical substance-specific limits, which are dependent on chemical analysis for enforcement, are therefore of limited use in authorising, and controlling the environmental consequences, of the discharge of complex chemical mixtures.

An evaluation of the Dutch complex mixture toxicity testing approach concluded (*inter alia*) that:

- methodologies for determining acute and chronic toxicity as well as bioaccumulation, mutagenicity and persistence do exist although not all of these are internationally standardised;
- a substance specific approach contributes very little environmental hazard information about effluents compared to the complex mixture toxicity testing approach; and
- it is not possible to draw generic conclusions as to the environmental hazards of effluents from different types of industry.

There is therefore a strong international move towards the use of toxicity testing of complex mixtures *in conjunction* with a substance-specific, chemical approach. It is important to note that

the use of toxicity testing is **not** an alternative to chemical testing and substance-specific limit values, but is a useful addition to the chemical approach.

Toxicity-based ecological hazard assessment (TEHA) is an integrated approach to complex waste discharge assessment, which includes the USEPA approach to whole effluent toxicity (WET) testing, and is similar to the UK direct toxicity testing, and the Dutch complex effluent testing.

In the control of complex wastewater discharges, toxicity-based ecological hazard assessment (TEHA) enables better insight into the working of mixtures of known and unknown hazardous substances in complex wastewater discharges. As such, it may address some of the shortcomings of the substance-specific approach by providing a more complete picture of the ecological hazard of the discharge. TEHA can be applied to a wide variety of discharges including industrial and treated sewage point discharges as well as to localised diffuse sources. The implementation of this approach needs to be phased-in on a priority basis in order to facilitate skills development. Information on known substances in discharges, data from experimental and pilot implementation data, as well as information gained from biomonitoring, will be used in this prioritisation.

Ecological hazard can be brought about by a number of root causes, which are referred to as parameters of the hazard. Each of the parameters is assessed by a different test. Consequently, TEHA consists of a battery of tests to be performed on a sample of the effluent to show up potential adverse effects. The tests selected at this stage is representative of those commonly available but the selection needs to revisited periodically to ensure that the most cost-effective selection of tests is maintained.

The suite of toxicity tests and techniques, for the evaluation of the proposed TEHA parameters, are either standardised or at least well known enough internationally to consider their application to wastewater discharge assessment in South Africa. The problem in the South African context is capacity in terms of skills. With the exception of two tests, the local capacity in performing the TEHA tests is not sufficient to allow a full implementation of this methodology. If sufficient stimulus for capacity building is provided, a full implementation could probably happen within five years.

However, both the TEHA parameter selection and the techniques for their assessment come from a dynamic research field and it would be necessary to keep up to date with research. Locally, development might focus on three themes:

1. increasing environmental realism of tests;
2. improving the affordability of realistic ecological hazard assessment; and
3. capacity building.

Development would require a co-operative effort and commitment from the public and private sectors as well as academia.

DWAF is in the process of preparing a document which aims to clearly describe how applications to discharge complex wastewater to water resources will be assessed and authorised . The document will link closely to the Government Gazette No. 20526 (October 1999); No 1191

"General authorisation in terms of Section 39 of the National Water Act (No. 36 of 1998)". The General Authorisation (GA) specifically excludes the discharge of complex industrial discharges, and the guideline will provide a procedure for dealing with complex wastewater (Schedule 3: 3.7 (1) (a) (iii)). The finalised guideline will apply to any discharge which reaches a surface water by any means, and which has more than 10% by volume complex industrial wastewater, as defined in the GA.

Definitions

Hazard	Having the potential to cause (often a specific) undesirable effect.
Risk	The likelihood of experiencing a specific undesirable effect.
Parameter	One of a set of measurable factors that define a system and determine its behaviour (American Heritage Dictionary of the English Language, 1996).
Complex waste discharge	A discharge of wastewater of which the composition and/ or impact cannot be assessed. In the present context this is assumed to be equivalent to the term "complex industrial wastewater" discharge as defined in regulations under the National Water Act of 1998.

4.3.2 A summary of the proposed tiered approach

Level 1

This is the screening level. Standard laboratory tests are used, and the wastewater is diluted by a laboratory medium in the test procedure. If the tests show that the wastewater does NOT pose a hazard, the least onerous level of licence can be granted.

If the screening tests identify that the wastewater DOES pose a hazard, the discharger has the option of identifying and reducing the source of toxicity in the mixture - or undertaking a level 2 test which will investigate the extent to which the chemistry of the receiving water will mitigate the toxicity of the wastewater.

Level 2

At this level, more tests are required, and tests are repeated using receiving water to dilute the effluent. If the tests now show that the wastewater does NOT pose a hazard, a licence can be granted which takes account of the mitigating effect of the natural water chemistry. However, as this can be variable - the licence and monitoring requirement will be more onerous.

If these level 2 tests identify that the wastewater STILL poses a hazard, the discharger has the option of identifying and reducing the source of toxicity in the mixture - or undertaking a level 3 test which will take account of the dilution capacity of the receiving environment.

Level 3 A simple dilution model is applied, using information on the hydrology of the receiving water resource. If the tests now show that now the wastewater does NOT pose a hazard, a licence can be granted which takes account of the mitigating effect of the natural water chemistry and the dilution capacity of the receiving water. However, as this can be even more variable and therefore more risky - the licence and monitoring requirement will be still more onerous.

If these level 3 tests identify that the wastewater STILL poses a hazard, the discharger has the option of identifying and reducing the source of toxicity in the mixture - or undertaking a level 4 test which will take account of the site-specific resource quality objectives that have been set for the resource. In addition, the individual resource will be considered in the context of the wider catchment.

Level 4 In addition to the end-of-pipe assessment, instream conditions and objectives, as well as a catchment scale, are taken into account. If the level 4 tests now show that now the wastewater does NOT pose a hazard, a licence can be granted which takes account of the mitigating effect of the natural water chemistry, the dilution capacity of the receiving water, and the instream resource quality objectives. However, as this can be even more variable and complex, and therefore more risky - the licence and monitoring requirement will be still more onerous.

If these level 4 tests identify that the wastewater STILL poses a hazard, the discharger has the option of identifying and reducing the source of toxicity in the mixture - or undertaking a comprehensive, site-specific, ecological risk assessment.

Level 5 An environmental risk assessment provides managers with a comprehensive basis on which to evaluate the risk associated with the discharge of the wastewater. The ERA will identify conditions for discharge, which would then be incorporate into the licence and monitoring conditions, or identify that the wastewater should not be discharged.

4.3.3 Conclusion

The method development and results from this project have made a significant contribution to the routine assessment of water quality within the ecological Reserve. The project team has worked closely on the development of a discussion document and supporting technical guideline for the implementation of toxicity testing in the source directed control of complex wastewater discharge and impact. The methods for the use of riverine organisms in toxicity testing are included in the site-specific (Level 5) assessment of complex wastewater discharges.

CHAPTER 5

OPPORTUNITIES, RECOMMENDATIONS AND CONCLUSIONS

5.1 INTRODUCTION

In 1994 Roux summarised the status of applied aquatic toxicology as follows:

> "To cope with increasing demands placed on the quality and quantity of aquatic resources, efficient management tools are continually required. Recent developments in environmental monitoring indicate the importance of incorporating biological indicators in assessment programmes. Aquatic toxicology has consequently become an important monitoring and regulatory science. Applications of aquatic toxicity testing include: deriving water quality criteria; toxicological evaluations of whole effluents and receiving waters; and the estimation of ecological risk. Toxicity testing can potentially play a significant role in improving water quality in years to come, especially through its application in effluent regulation. Currently however, few environmental laboratories in South Africa have the required expertise and facilities to carry out a representative range of toxicity tests. Training and funding are required to build the capacity for the necessary developmental research, before toxicity testing can routinely be implemented." Roux (1994).

It has proved to be difficult to realise this vision. Some progress is evident: Slabbert et al. (1998a; b) published appropriate methods; international ecotoxicity databases were used to derive aquatic ecosystem guidelines (DWAF,1996); and toxicology laboratories are more common (accredited laboratories at the Institute for Water Quality Studies (DWAF) and Rand Water). However, in 2002 there is still no routine use of toxicity data in licensing and the application of source directed controls.

This project has however been part of key steps towards the realisation of this objective:

- In May 2000 the project team assisted the WRC in the organisation of a workshop for role-players where DWAF expressed a commitment to move towards implementation;
- in 2001 the aims and products of this project were shifted so that project team members worked with DWAF personnel in the production of a discussion document and a technical guideline for the use of toxicity testing in the management of complex wastewater discharges;
- in 2002 DWAF will release the discussion document and the technical guideline, and after Departmental discussions, a pilot implementation will be conducted;
- in 2001 Ecological Risk Assessment guidelines were published (Claassen et al., 2001), citing the central role of toxicity testing in certain site-specific ERAs;
- in 2002 DWAF will initiate a national programme for instream toxicity monitoring.

Ecotoxicology can also contribute to resource directed measures:

- In 2002 DWAF will publish an ecological Reserve assessment manual which will recommend the inclusion of toxicity assessments within the water quality aspects of ecological Reserve assessments, and
- the national instream toxicity monitoring programme will also contribute to RDM.

This means there are real opportunities for using ecotoxicology in the implementation of both RDM and SDC. However, current initiatives are only the first steps. In both the RDM and SDC arenas there are areas of research that are critically important to successful implementation.

5.2 RECOMMENDATIONS

Some of the recommendations will be taken up as research objectives in the period 2002-2005. Where this is the case the lead institution is listed in brackets at the end of the paragraph.

5.2.1 Environmental water quality and the use of toxicity testing in ecological Reserve assessments

General research requirements

The following areas of water quality within ecological Reserve assessment require specific research attention:

- The frequency and manner of water quality monitoring restricts the kinds of datasets that are available. At present, only concentration (magnitude) is considered, whereas frequency and duration also need to be taken into account. There is a need to see if water quality RQOs can be developed in a way which matches hydrological assessments in taking account of magnitude, duration and frequency of concentrations. To do this, the following actions are necessary: evaluation of current water quality databases for the development of acceptable time-series data for selected water quality variables; assessment of the applicability of a risk-based approach within the limitations of existing water-quality data; and recommendation of optimal water quality monitoring in the light of emerging data requirements. Toxicity data can be used in time-series generation as they allow chemical stressor-response relationships to be quantified (O'Keeffe et al., in press).(CAT-IWR).

- Ecological Reserve assessments rely heavily on expert opinion and judgement (King et al., 2000; in press) and in order to capture expert opinon and facilitate a systematic and consistent application of methods, an Ecological Reserve Decision Support System (DSS) is being developed (Hughes, pers. comm). There is a need to integrate water quality data, tools, techniques and methodologies within this DSS. To do this, the following actions are necessary: Delphi code needs to be written that will allow the technical integration of existing water quality data, tools, techniques and methodologies within the DSS; water quality data formats need to be specified, qualitative decision criteria need to be recorded. (CAT-IWR)

- Nutrient assessments require particular attention and nutrient criteria need to be selected (e.g. total phosphate, dissolved phosphate, total nitrogen or a ratio of two or more nutrients). The assumption in the Reserve that the reference condition in all ecoregions is equal to or less than the Target Water Quality Range in the South African Water Quality Guidelines for aquatic ecosystems (DWAF, 1996) needs to be validated, and a better nutrient evaluation protocol needs to be developed. Nutrients are not toxic and it may be that zooplankton and invertebrate feeding behaviour or rates should be used as indices of health. (Freshwater Research Unit, University of Cape Town: FRU, UCT).

- The link between chemical, biotic, and toxicological data needs to be further developed and an assessment protocol written. The method for relating biomonitoring scores to ecological health classes needs to be formalised and recorded. (FRU, UCT).

- Methods for monitoring, assessing and relating turbidity and suspended solids levels to biotic responses and ecosystem health classes, need to be developed.

Salinity tolerance testing

South Africa is not alone in having salinity as a major water quality issue. In Australia the Government has pledged a large proportion of its research and water quality management funding to solving problems relating to salinisation (Nielsen and Hillman, 2000). South Africa has taken the international lead in the development of integrated methods for the quantifying of environmental flow requirements (King et al., 2000; in press).

The work presented in this report suggests that these two issues are linked. Dissolved ion concentrations and ratios in freshwater result in the first place from geology, and provide the basic chemical "fingerprint" of the water. Environmental water chemistry finally results from a combination of organic and inorganic processes. Different salts elicit different tolerance responses, and the tolerances differ regionally and between taxa.

Alternative methods for using salt tolerances in RDM, and methods for using salt tolerance data in SDC will depend on species- and site-specific data. It is important that the CAT-IWR salt tolerance database be used to erect hypotheses to 1) plan further tolerance experiments, and 2) erect hypotheses and test alternative approaches to modelling the relationship between salt tolerances and ecosystem health classes. (CAT-IWR).

Instream toxicity testing

The Olifants River study demonstrated that biomonitoring can reveal chemical stress not revealed by conventional chemical monitoring, and that this could then be linked to measurable instream toxicity (Palmer and Rossouw, 2001). It is important now to investigate sub-lethal effects, and to develop cost-effective methods for the evaluation of long-term sub-lethal effects. (CAT-IWR).

DWAF has already indicated a plan to implement instream toxicity monitoring.

Ultimately the aim would be to link an understanding of invertebrate physiology, with the fate and effects of chemical mixtures in the environment, and the ecology of freshwater systems.

Integration of environmental water quality and quantity requirements

It was obvious at the Environmental Flows for River Systems & Fourth International Ecohydraulics Symposium hosted by Southern Waters Ecological Research and Consulting, held in Cape Town during March 2002, that there has been little international attention paid to the integrated assessment and management of environmental water quality and quantity. It would be timeous for the South African water quality Reserve specialists to collaborate on a book which focusses on environmental water quality methods and applications. (CAT-IWR).

5.2.2 Environmental water quality and whole effluent toxicity (WET) testing

There is a strong likelihood that DWAF will implement complex industrial effluent management procedures using whole effluent (WET) toxicity testing, with a tiered approach called TEHA

(Toxicity-based Ecological Hazard Assessment). For the envisaged Level 5 of TEHA, WET testing will need to be more sophisticated, with efficient, effective methods of assessing the sub-lethal, long-term ecological effects of complex mixtures. Ongoing work on sub-lethal measures, and the relationship between dilution and ecological health class will be essential. It will be important to develop methods and protocols to ensure that TEHA becomes part of routine ecological risk assessments of water quality impacts. (WRC Ecotoxicology Programme).

5.3 CONCLUSIONS

With regard to salt tolerances: riverine organisms are more tolerant of salt concentrations than are generally experienced under natural freshwater conditions. However, high salinities often have anthropogenic causes and can have lethal and/or sub-lethal effects. *Daphnia* are generally more sensitive to increased salt concentration than most freshwater invertebrates, but possibly not as sensitive as first instar or juvenile stages (e.g. freshwater shrimp neonates). The results of *Daphnia* tests are subject to the application of safety factors so as to ensure protection. It therefore makes economic sense to use *Daphnia* as the freshwater invertebrate in screening tests; riverine invertebrates for evaluations of intermittent, short term, high salinity events; and riverine invertebrates (using both juveniles or early instars and/or sub-lethal measures) for evaluating site-specific effects of sustained elevations on salinity.

In 1992 there was no information on the tolerances of South African riverine macroinvertebrates to either single substances or complex mixtures. There have been considerable developments in ecotoxicology since then: CAT-IWR has a South African riverine invertebrate tolerance database; the WRC has an Ecotoxicology research programme; and DWAF has plans for the active use of ecotoxicology in both RDM and SDC. This project has contributed both to the development of RDM and SDC water quality assessment methods, and to the management and policy frameworks which direct the use of ecotoxicology.

Although further method development is required, there is also a need for a period of critical review and consolidation, particularly with regard to the generation, curation, and publication of tolerance data of South African invertebrate taxa. The science of ecotoxicology is currently well-placed to contribute to environmental quality management both in South Africa and internationally.

CHAPTER 6

COLLABORATION AND CAPACITY BUILDING

6.1 COLLABORATION AND CONTRIBUTIONS

6.1.1 Research collaboration and contributions

The project team have collaborated with a wide range of people and institutions, but there are researchers who have more than collaborated - they have significantly contributed to this report. They are:
Dr Sebastian Jooste, DWAF , IWQS: critical discussion of all aspects of the project, development of an alternative use of salinity tolerance data in ecological Reserve assessments, and co-writing of the technical guideline for TEHA;
Dr Heather Malan FRU,UCT: critical discussion of all aspects of the project, and development of a model which related flow to instream salt concentrations;
Mr Nico Rossouw, Ninham Shand: critical discussion of all aspects of the project, and co-development of all the water quality Reserve methods; and
Dr Dirk Roux, CSIR: evaluation of the project products and the development of an objectives hierarchy and adaptive management plan for future research.

Our graduate students have stimulated our thinking and contributed data and reports:
Mrs Tandiwe Zokufa worked on the acute effects of textile and pulp and paper effluents (Zokufa et al., 2001), Appendix 3, and Ms Vicky Everitt worked on complex industrial effluents (Everitt, 1999). Mrs Heather Davies-Coleman began her doctoral work on the reproduction of a freshwater limpet which allowed her to evaluate the sub-lethal effects of textile effluent. Honours students Ms Nolwazi Mkize, Mr Lindela Tshwete, Ms Sandy Dlamini and Ms Sekiwe Mbande all did projects which contributed to this research.

Institutions with which we have collaborated:

- Rhodes University: Department of Zoology and Entomology, Department of Geography, Department of Journalism, Department of Biochemistry and Microbiology, Department of Chemistry, Department of Ichthyology and Fisheries Science, Department of Botany (teaching and student supervision)
- DWAF: Institute for Water Quality Studies; Directorate Water Quality Management; Directorate Strategic Planning (co-funding, see Section 6.2)
- University of Cape Town - Freshwater Research Unit (collaboration on RDM water quality methods)
- University of Transkei (collaboration on approaches to turbidity)
- Unilever UK and Lever Ponds South Africa (investigation of the effects of detergents in stream - where dissolved detergent powder was used as a complex wastewater.)
- Richards Bay Minerals (contributions to the management of sulphate-rich waste water)
- SASOL (biomonitoring and ecotoxicity testing)
- CSIR (development of Ecological Risk Assessment (ERA) guidelines, (Claassen et. al., 2001) and the role of ecotoxicity results in ERA).

6.1.2 Co-funding

At the start of this project there was no routine, developed method for assessing water quality within ecological Reserve assessments, and the development of both the methods and data for pilot application of the methods were funded from a range of projects (Table 6.1). This project has also worked to develop methods and standard procedures that will allow ecotoxicology to

be used in SDC. In these endeavours, the two main funders were the WRC and DWAF-IWQS. The products of all the associated projects are listed in Table 6.1; they are linked and are mutually supportive.

TABLE 6.1

THIS PROJECT, "USE OF RIVERINE INVERTEBRATES IN APPLIED AQUATIC TOXICOLOGY AND WATER RESOURCE-QUALITY MANAGEMENT" WAS FUNDED BY THE INSTITUTE FOR WATER QUALITY STUDIES (IWQS, DWAF) AND THE WATER RESEARCH COMMISSION (WRC). PROJECT FUNDS FROM THE WRC CONTRIBUTED TO SALINITY TOLERANCE TESTING FOR THE OLIFANTS RIVER ECOLOGICAL RESERVE ASSESSMENT, AND POST GRADUATE WHOLE EFFLUENT TOXICITY (WET) TESTING STUDIES OF TEXTILE, PULP AND PAPER AND COMPLEX INDUSTRIAL EFFLUENTS. THE IWQS, DWAF PRODUCTS WERE A STANDARD PROTOCOL FOR SINGLE SUBSTANCE TOXICITY TESTING IN FLOWING WATER, AND AN ASSESSMENT OF THE METHOD FOR WET TESTING IN FLOWING WATER. PRODUCTS FROM THREE OTHER WRC PROJECTS HAVE ALSO BEEN USED IN AN INTEGRATED MANNER. SDC - SOURCE DIRECTED CONTROL; CWD - COMPLEX WASTEWATER DISCHARGE. CAT-IWR - CENTRE FOR AQUATIC TOXICOLOGY, IWR

Funder	Project	Product from project	Applications:			
			Ecological Reserve Methods	Flowing water toxicity protocol	SDC of CWD	Instream toxicity monitoring
WRC	Use of riverine invertebrates in applied toxicology	CAT-IWR database & variability	✔			✔
		Olifants River Reserve	✔			
		Textile chronic WET testing			✔	
	Sabie and Buffalo Rivers	Textile & Pulp/paper CDW acute WET testing			✔	
	Vaal River WET tests	CWD WET testing			✔	
	Water quality Reserve	Breede River Reserve	✔			
IWQS-DWAF	Use of riverine invertebrates in applied toxicology	Methods for riverine test organisms: single substances	✔	✔		
		Methods for riverine test organisms: complex mixtures		✔	✔	
		SDC of complex wastewater discharges			✔	

6.2 CAPACITY BUILDING

Where designated groups:	black people (i.e. black, coloured or Indian people), women, and disabled people

6.2.1 Capacity building within designated groups

Employment and development of staff from designated groups

Mr Phehello Mahasele joined CAT-IWR in March 2000. He has a BSc Honours degree in Leather Science and completed the CAT-IWR certificated course in Introductory Applied Aquatic Toxicology in May 2000. He was trained in laboratory methods required for the use of *Daphnia* spp. and indigenous riverine organisms in aquatic toxicity testing; participated in consulting projects; and was registered for an MSc on sub-lethal testing of complex wastewater discharge. Mr Mahasele suffered a tragic motor accident in July 2001, and was unable to work from then on. His MSc studies have been suspended.

Ms Michele Stewart was a research assistant in 2000 and 2001. Ms Stewart is a mature student, studying part-time, and as a severe epileptic can be considered disabled.

Funding and supervision of post-graduate students from designated groups

The following BSc Honours students have all completed courses and/or projects in applied aquatic toxicology: Mr Thabani Mlilo, Ms Nolwazi Mkize, Ms Sekiwe Mbande, Ms Nomahlube Sishuba, Ms Kim Wilkins, Ms Sandy Dlamini and Mr Lindela Tswhete.

Co-operative research ventures with previously disadvantaged institutions (PDI's)

Mr Bonani Madikzela, previously from UNITRA (but now with IWQS,DWAF) is undertaking tolerance testing of riverine invertebrates to elevated turbidity as part of his doctorate, which is being co-supervised by Dr Scherman.

Partnering staff from government departments

The project team has worked with the Institute for Water Quality Studies (IWQS, DWAF) to pilot a partnership programme where we have effected direct knowledge and technology transfer by interacting with selected IWQS staff. Mrs Tandiwe Zokufa and Mr Malesela Papo have both worked on ecological Reserve assessments with staff of CAT-IWR.

Community involvement

The project team was involved in an investigation of the effects of in-stream detergent use. Mr Malixole Soviti completed an MSc in January 2002 on community-use of detergents for in-stream washing. This work builds on WRC-funded community research conducted by Ms Nicole Motteux (Geography Department, Rhodes University).

6.2.2 Capacity building within designated as well as non-designated groups

Capacity-building of research personnel, industry and managing agencies from designated, as well as non-designated groups

CAT-IWR has a well-developed network of interactions with other researchers and practitioners in aquatic toxicology, and participate in the Aquatox Forum. We initiated a WRC-funded national workshop on applied aquatic toxicology and risk assessment and are involved in ongoing communication about toxicology with other researchers, DWAF and industry. We have developed course-ware in applied aquatic toxicology and in the 2 courses that have been offered, more than 50 % of the delegates were from designated groups.

Contracts

CAT-IWR is regularly involved in consulting work in the field of applied aquatic toxicology and environmental water quality. We are committed to using these opportunities for capacity building of our staff, students and associated colleagues. To date Mr Phehello Mahasele, Mrs Tandiwe Zokufa, Mr Malesela Papo, Mr Tobile Bokwe and Mr Bonani Madikizela have worked with us on CAT-IWR contracts in a capacity building relationship.

Lecturing

The project team contribute substantially to the Applied Freshwater Ecology semester course. The emphasis of the course is on natural resource protection and sustainable use and uses as its framework the environmental protection policy of the 1998 National Water Act. It is offered at third year level to students in the Departments of Zoology, Entomology, Environmental Science, Chemistry, Microbiology, Geography, Ichthyology and Fisheries Science, and Botany. Prof Palmer also lectures part of a Water Resource Management module, i.e. an honours level course taught in the Department of Geography.

The project team lectures a module on Applied Aquatic Toxicology to honours students registered in the Departments of Zoology and Entomology, Environmental Science and Geography.

The project team also teaches an intensive week of Applied Aquatic Toxicology to students registered for an MSc in Biotechnology at Rhodes University (Department of Biochemistry and Microbiology).

Masters and doctoral graduates and students

Heather Davies-Coleman	The growth and reproduction of the freshwater limpet *Burnupia stenochorias* (Pulmonata, Ancylidae), and an evaluation of its use as an ecotoxicological indicator in whole effluent testing. **Graduated 2002**
Supervisors	Prof CG Palmer and Prof AH Hodgson
Degree	PhD (Zoology) Rhodes University
Description	The growth and reproduction of *Burnupia stenochorias* was determined under laboratory and field conditions. These life

	history parameters were then compared to those exhibited by the animal experiencing chronic toxicity. Comparisons were also made with *Daphnia pulex* under similar conditions to the toxicity tests mentioned above.
Marius Claassen	The development of an Ecological Risk Assessment methodology for application in South African water resource management
Supervisors	Prof CG Palmer
Degree	PhD (Zoology) Rhodes University
Description	Ecological Risk Assessment (ERA) has an important role to play in the management of South African water resources. This project facilitated the development of an appropriate ERA tool for local conditions using a South African catchment to test the methodology.
Bonani Madikizela (University of Transkei)	The effects of suspended sediment on macroinvertebrates in the Umzimvubu River, Eastern Cape, South Africa.
Supervisors	Prof JH O'Keeffe and Dr P-A Scherman
Degree	PhD (Zoology) Rhodes University
Description	Soil erosion is one of the greatest problems facing land management in communal areas within the Eastern Cape. The extent to which the suspended sediment loads impact on the life history of the macroinvertebrate communities within the rivers of the region is poorly understood. This study will attempt to address this concern by quantifying the effects of suspended sediment on biota in the laboratory and field.
Markus Binder	An evaluation of recirculating stream designs for acute toxicity testing using two South African Ephemeroptera species exposed to sodium sulphate.
Supervisors	Prof CG Palmer and Dr P-A Scherman
Degree	MSc (Zoology) Rhodes University **Graduated 1999**
Description	It was demonstrated that salinity tolerance testing could effectively be done using small-scale rather than large-scale artificial streams.
Victoria Everitt	The use of indigenous macroinvertebrates and *Daphnia pulex* in acute toxicity testing.
Supervisors	Dr WJ Muller and Dr P-A Scherman
Degree	MSc (Zoology) Rhodes University **Graduated 1999**
Description	It was demonstrated that using both riverine and *Daphnia* spp in applied ecotoxicolgy is an advantage.
Malixole K Soviti	Investigating the impact of the instream use of detergents on water quality in the upper Kat River valley, Eastern Cape.
Supervisors	Prof CG Palmer and Prof KM Rowntree
Degree	MSc (Geography)

	Graduated 2002
Description	Laundry related activities are routinely carried out in the streams of the upper Kat River valley. Detergents were found to have a significant impact on the chloride concentrations, electrical conductivity and turbidity of the river water. These effects had almost completely dissipated 1 km from the laundry site.
Zanele Mabaso	Development of a biomonitoring methodology for two rivers in the Eastern Cape.
Supervisors	Dr P-A Scherman and Dr WJ Muller
Degree	MSc (Zoology) Rhodes University
Description	The project is in its early stages, but the biological and physical indices developed to monitor the two rivers are expected to be widely applicable within the Eastern Cape.
Phehello Mahasele	Sub-lethal and chronic toxicity responses of the freshwater shrimp *Caridina nilotica* (Decapoda: Atyidae) to sodium sulphate
Supervisors	Dr P-A Scherman & Prof CG Palmer
Degree	MSc (Zoology) Rhodes University
Description	**Degree suspended July 2001 due to motor accident.**

Short courses

Unilever course in Applied Aquatic Toxicology

The course is the major CAT-IWR capacity building initiative, to which all staff contribute. It is an intensive one-week course, aimed at mid-level managers and staff from regulatory agencies, consultancies and industry, and includes a balance of lectures and practicals in order to provide a comprehensive overview of the use of aquatic toxicology in water resource management. The course in 2001 was co-ordinated by Dr Nikite Muller, and took place in May. Participants included 11 Rhodes MSc Biotechnology students (for whom the course is a compulsory module); 4 CAT-IWR honours students (for whom the course is followed by four intensive 3-hour tutorials); and 6 professionals (mainly from the regulator - DWAF, and industry). Half the delegates were female, and half from previously disadvantaged groups.

Biomonitoring course

The *National Short Course on the role and use of Aquatic Biomonitoring* has been hosted by the IWR since 1999 and is co-ordinated by Drs Patsy Scherman and Nikite Muller. The aim of the course is to provide an understanding of the concepts of different biomonitoring techniques and demonstrate how they can be used in water resource management. The course requires the coordination of more than 20 lecturing staff, including members of CAT-IWR, and management of a timetable which includes lectures and both field and laboratory practicals. The course is attended mostly by staff from regulatory agencies, consultancies and industry, although students from various tertiary institutions have attended. In 2001 there were 35 delegates.

6.3 PUBLICATIONS, REPORTS AND PRESENTATIONS PRODUCED BY PROJECT TEAM MEMBERS JULY 1998 - JUNE 2001

6.3.1 Full-length papers in specialized technical journals and books

Gerhardt A and Palmer C G (1998) Copper tolerances of *Adenophlebia auriculata* (Eaton) 1884 (Insecta: Ephemeroptera) and *Burnupia stenochorias* Cawston 1932 (Gastropoda: Ancylidae), in indoor artificial streams. *The Science of the Total Environment* **215:** *217-229)* .

Palmer CG (1998) Setae and microtrichia - structures for fine particle feeding in aquatic larvae. Chapter 12. Pages 289-302 In: *Microscopic Anatomy of Invertebrates, Vol 11A Insecta.* Eds. F Harrison and M Locke. John Wiley and Sons, New York.

Palmer CG (1999) The application of ecological research in the development of a new water law in South Africa. *Journal of the North American Benthological Society* **18:** 132-142.

Palmer CG, Peckham B and Soltau F (2000) The role of legislation in river conservation. Pages 475-491. In: *Global Perspectives on River Conservation: Science, Policy and Practice.* Edited by P J Boon, BR Davies and G Petts. John Wiley and Sons, UK.

6.3.2 Reports

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APPENDIX 1

ACUTE TOXICITY TEST PROTOCOL AND TOXICITY DATABASE DEVELOPMENT

1.1 A PROTOCOL FOR ACUTE TOXICITY TESTING USING SELECTED RIVERINE INVERTEBRATES IN ARTIFICIAL STREAM SYSTEMS: VERSION 1.0

The protocol was published in 2000 by the Department of Water Affairs and Forestry (DWAF), and describes acute toxicity testing using riffle-dwelling freshwater indigenous invertebrates as test organisms in recirculating artificial stream systems. Methods are modelled on, and modified from, the standard methodologies described in the EPA manual for acute toxicity testing (US EPA, 1993), and the toxicity testing methods described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1992). The protocol therefore provides specific formalised instructions and a standard method for the use of riverine invertebrates as aquatic toxicity test organisms.

Developmental research was carried out by the Centre for Aquatic Toxicology of the Institute for Water Research, Rhodes University in Grahamstown, South Africa, and funded by the Water Research Commission. The protocol was prepared by staff of the CAT-IWR, and authored by Scherman and Palmer.

The following information is contained within the protocol:

- Context and scope of the protocol
- Application in water resource management
- Background
- Types of artificial streams
- Facilities and equipment
- Experimental design
- Acute toxicity test procedure
- Data processing
- Database design
- Quality assurance
- Recommended report structure
- References

The protocol is available on request from CAT-IWR or Dr Anneli Kühn of the Institute for Water Quality Studies, DWAF.

1.2 CAT-IWR TOXICITY DATABASE DEVELOPMENT: VERSION 2.0

1.2.1 Introduction

A strong need was perceived to develop a toxicity database at CAT-IWR, as over the past few years a large amount of data was collected by the Centre. Previously it was stored in spread sheets (Quattro Pro) and was not always easily accessible. Data manipulation was also difficult. This database represents mainly indigenous invertebrate toxicity data, but has been extended to include data of standard laboratory organisms (e.g. *Daphnia pulex*) and other aquatic organisms (e.g. Tilapia *Oreochromis mossambicus*).

The database has been developed in ACCESS and is linked to the Microsoft Office suite of programmes (i.e. Excel, Microsoft Word), which allows their use for data analysis. The database has a range of query tables, facilitating data manipulation and comparison. This is an update from the database version in *A protocol for acute toxicity testing using selected riverine invertebrates in artificial stream systems*. Changes or alterations to the database, Version 2.0, are underlined and old names are placed in brackets.

The database has been designed by Mr Dave Hanton of Eastern Cape Computer Consultants.

1.2.2 Database structure

Access to the database

To access the database, an *IWR* option should be chosen from the main menu and then an *Experiment* option. It will bring up the *Main Details* screen. Other options are also available from this menu and they are used for inputting such parameters as:

Area
Analytical laboratory name
Phylum
Class
Order
Family
Genus
Organism (a species name)
Laboratory name (name of the laboratory where the experiment was conducted)
Parameters (water chemistry data)
Experimental vessel type (previously *Stream type*)
Toxicant
Units (for electrical conductivity)

Input of new entries or conducting a search can be done by clicking on the appropriate button.

Experiments Report and *Analysis* buttons are also linked to this menu. Their function will be explained in Section 1.2.4.

The ***Main Details*** screen gives information about experimental organisms (i.e. species name, organism source), the river from which organisms were collected (i.e. name, collection site description, water parameters), toxicant used for the experiment, starting date and duration of experiment, type of experimental vessel (e.g. channels, buckets, beakers) and number of vessels used. The *River* box is linked to the *Area Code* and the *Organism* box is linked to *Phylum, Class, Order, Family* and *Genus*, thus allowing data screening at these levels. A box to record *GPS* readings is available. The *Main screen* allows access to all other screens defining experimental conditions (Fig. 1).

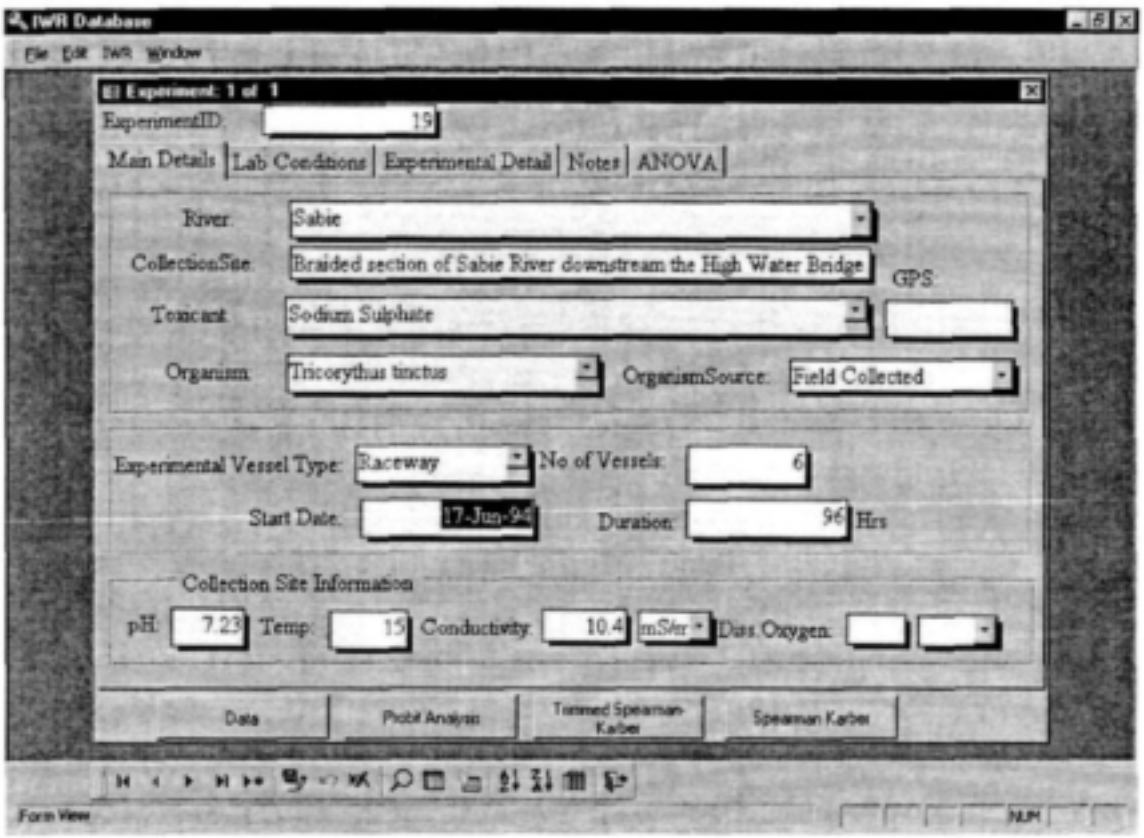

Figure 1
The Main Details screen.

The ***Laboratory Conditions*** screen describes the following information concerning the laboratory, such as:

Light regime i.e. a day/night cycle in hours

Light source i.e. biolux, fluorescent, natural, or a combination of these

Laboratory type i.e. permanent, semi-permanent, permanent site-specific, semi-permanent site-specific, controlled environment laboratory

Laboratory name

Daily laboratory temperatures recorded during the experiment are listed in the table (Fig. 2).

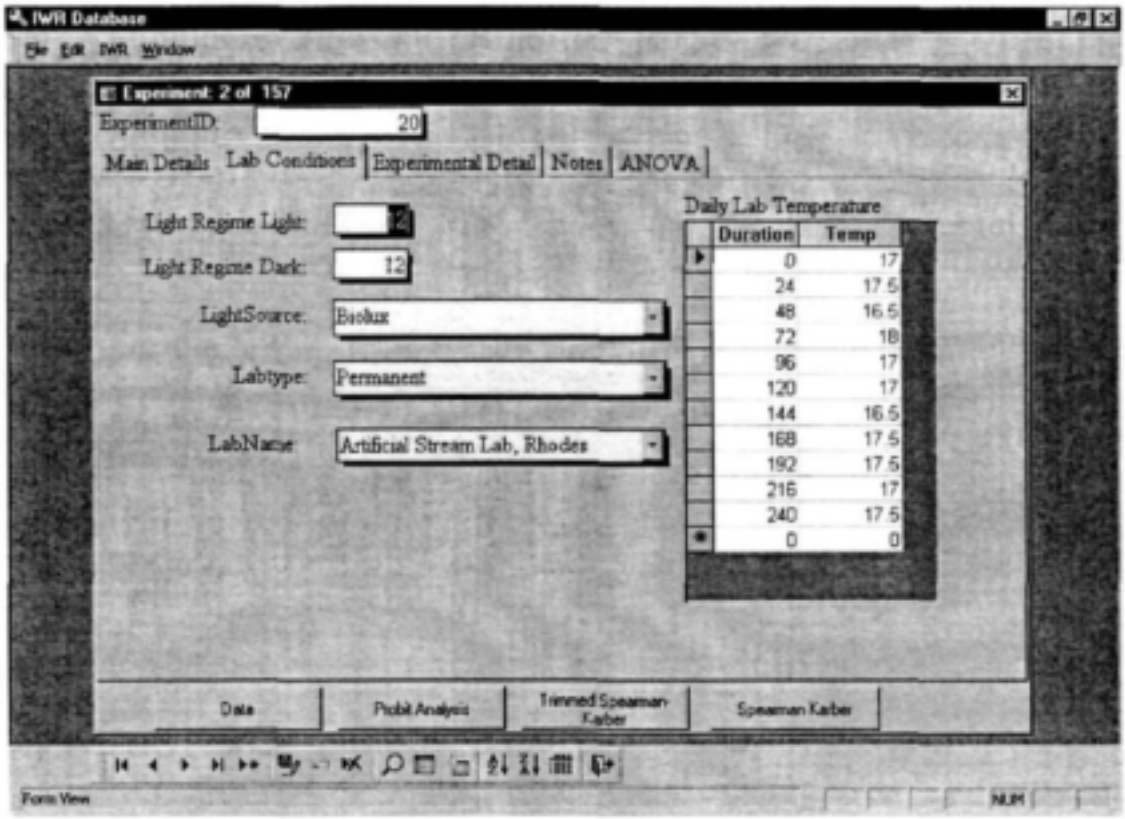

Figure 2
The Laboratory Conditions screen.

The ***Experimental Details*** screen describes information regarding the experiment, such as:

Acclimation time in hours

Diluent i.e. de-chlorinated tap water, river water, rain water, or reconstituted water

Number of concentrations of the toxicant tested

Experimental design i.e. replicated definitive, unreplicated range-finding, unreplicated regression, or replicated regression

Number of replicates

Test type i.e. recirculating, static, flow-through, bubble pot

End-point i.e. mortality, moulting or behavioural

Analytical laboratory name i.e. name of laboratory conducting the chemical analysis

Acclimation mortality in percentage, records the highest mortality during acclimation (Fig. 3).

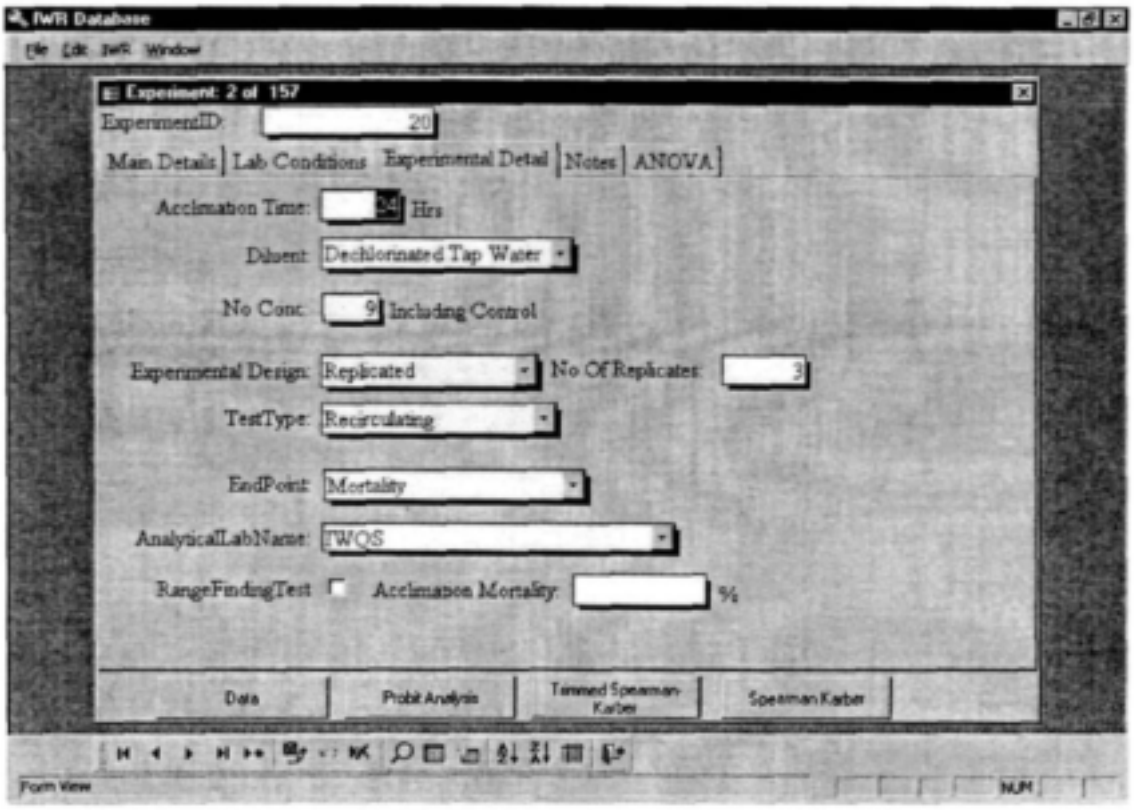

Figure 3
The Experimental Details screen.

The ***Notes*** screen records any additional information regarding a particular experiment, e.g. time when experimental medium was changed, or when samples for water quality analysis were taken.

The ***Data*** screen can be accessed from any of the above-mentioned screens. This screen contains experimental data. All data are entered per concentration. If an experiment was replicated, the *replica number* should be entered. At the present moment the *Data* screen is designed only for mortality as an end-point. *Mortality data* are entered after elapsed time periods. After mortality entries have been completed, the database calculates *Cumulative mortality*, and once *Live number* of organisms has been entered, the database calculates *Total Organisms* number and *% Cumulative Mortality*.

All parameters that are measured for the toxicant concentration are included in the *Laboratory Data* table. This table allows the inputting of the following parameters:

pH
temperature, ° C
electrical conductivity, mS/m
dissolved oxygen, mg/ℓ
nitrite, mg/ℓ
phosphate, mg/ℓ
nitrate, mg/ℓ
ammonium, mg/ℓ

The laboratory data are entered after elapsed time periods (Fig. 4).
Averages of laboratory data are available from the *Query tables*.

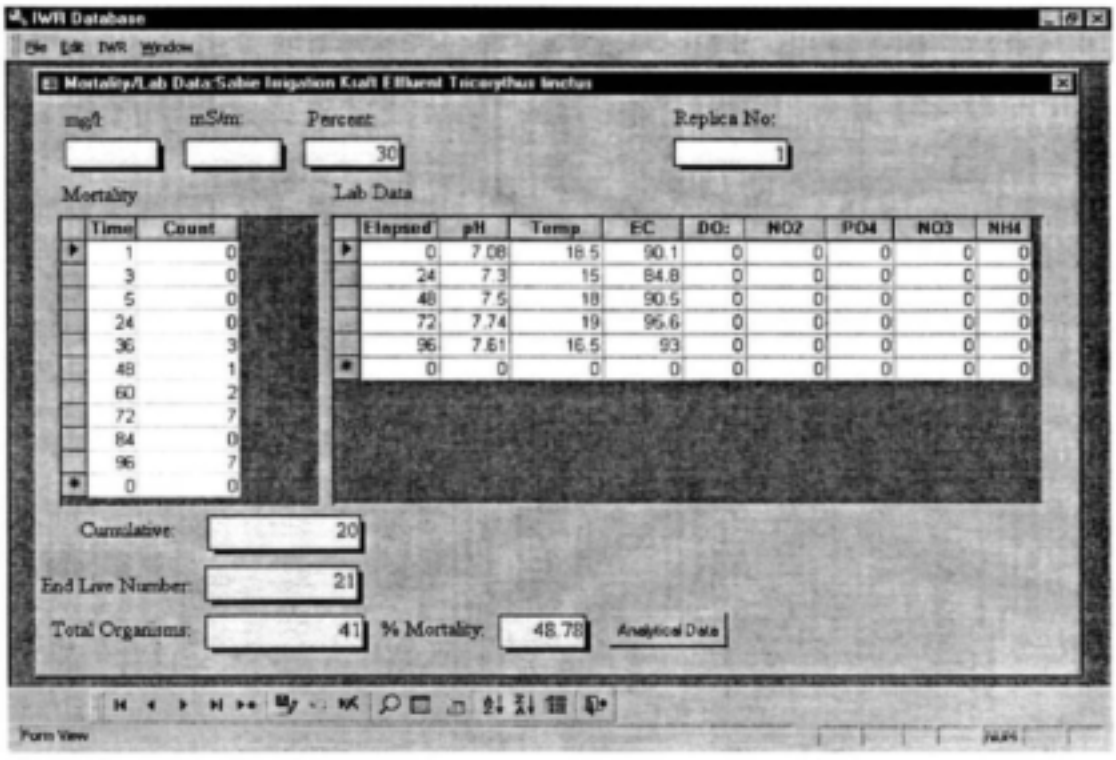

Figure 4
The Data screen.

Along the bottom of the *Data* screen is the *Analytical data* box, which allows access to the ***Analytical Data*** screen. Data from the water chemistry analysis conducted by the analytical laboratory is entered on this screen. Data will be entered per concentration per elapsed time periods. All results are entered in mg/l (Fig. 5).

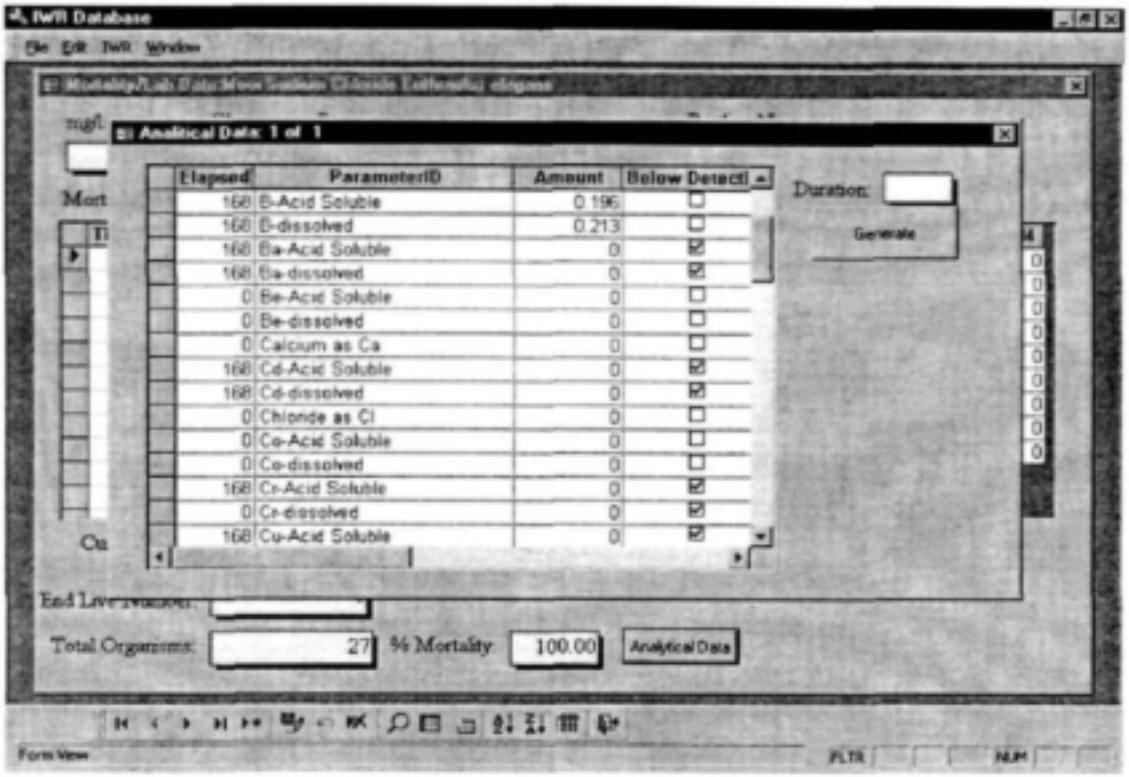

Figure 5
The Analytical data screen.

Statistical methods used in this database for the data analysis are ***Probit analysis program (Probit)***, ***Trimmed Spearman-Karber method (TSK)***, ***Sperman-Karber method (SK)*** and ***ANOVA***. The results of these analyses can be accessed from the *Main Details* screen by clicking on the appropriate button. Once results for these programmes have been completed, they can be entered into the database. As these are DOS-based programmes, they cannot be operated through the database. Statistical analysis data are entered per concentration per replica for the chosen time periods. If a statistical method cannot be used for a particular experimental data set, this fact should be reflected in the *Notes* section. Results for the statistical analysis are linked to the *Query tables*.

1.2.3 Entering new data

Before entering data for a new experiment, the data capturer must ensure that the organism name, river name, toxicant name and vessel type has been entered into the database. The database has a facility which allows one to copy information from one experiment to another, which is very important in cases where multiple species have been used per experimental vessel. The data are entered per species, but when more than one species is used per experimental vessel, these experiments should be treated as separate.

1.2.4 Conducting a search

The database has a search facility, called ***Find***, which allows one to seek information on either a single parameter from any of experimental screens (e.g. an experiment ID number, a river name, a toxicant name, an organism name, a vessel type, a test type, a light source) or any combination of these parameters. The database will show a list of experiments that will match with a required request. All chosen experiments can be opened for a detailed search.

Query tables

This is a new addition to the database. A range of query tables has been developed for data manipulation and accessing comparative information. *Query tables* can be accessed by choosing the *Analysis* option from the *IWR* menu. Another option available on the *IWR* menu is *Experimental Report*. New query tables could be developed later should the need arise. Listed below are query tables available at the moment:

- ***Experimental report*** gives general information about all experiments that are stored in the database, i.e. an experiment ID number, a river name, a starting date, a toxicant name, a species name.

- ***Average Parameters***: Three query tables fall under this name, as toxicant concentration can be entered into database using the following units: *mg/l*, *mS/m* and *percent*. *Average Parameters* tables have been developed for each of these units respectively. They show average pH, temperature and electrical conductivity for a chosen experiment for the duration of the experiment.

- A ***Report Form*** screen is also linked to the *Analysis* option. Along the bottom of this screen are listed *Cumulative Mortality*, *Probit Data* and *TSK*, thus allowing one to see either Cumulative Mortality and Percent Cumulative Mortality results, or Probit analysis results, or Trimmed Spearman-Karber results for any of the following: a particular experiment ID number, or genus, or species, or river, or toxicant, or vessel type, or any combination of these. If an asterisk (*) is chosen, the database will show the results for all of the above-mentioned parameters. See Fig.6.

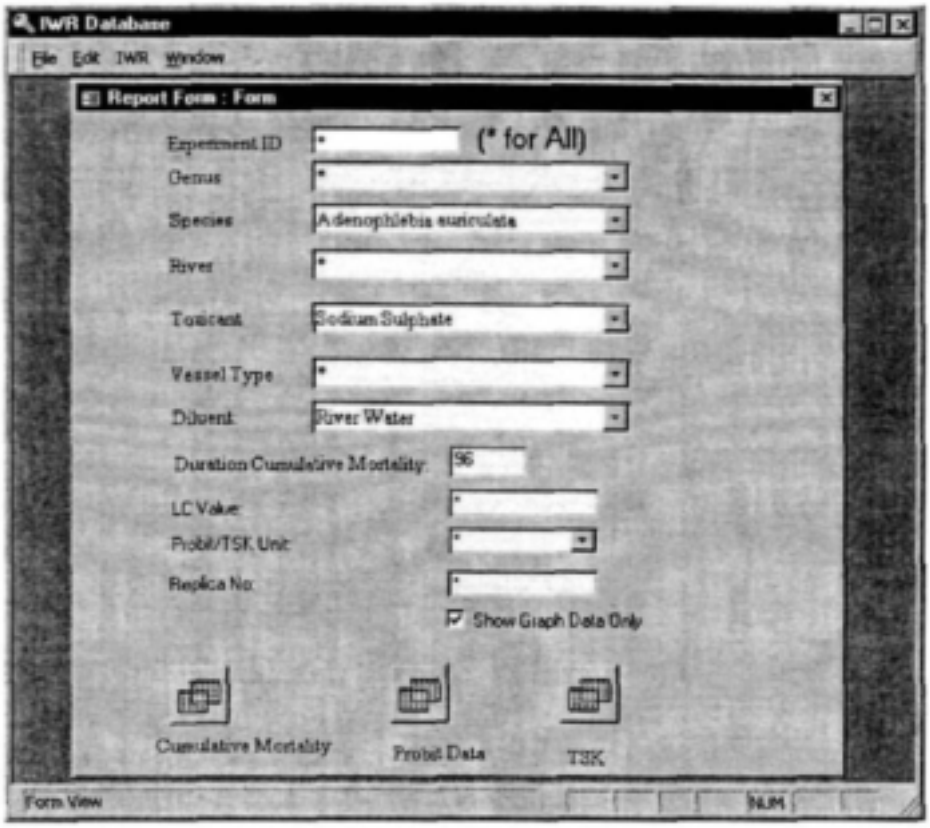

Figure 6
The Report Form screen.

- A table of ***Cumulative Mortality*** shows the cumulative mortality and percent cumulative mortality results for time elapsed and contains a variety of other parameters, such as the experimental ID number, a species name, a river name, a toxicant name, a vessel type, a diluent type, etc.. Data from the *Report Form* or from any other query table can be exported from the database to Excel files or any other Microsoft Office programme files. See Fig. 7.

- If a ***Show Graph Data Only*** option has been chosen together with a *Cumulative mortality* option, the database will bring up the table of *Percent cumulative mortality*, thus allowing one to export this table into an Excel file and to use it for building a concentration-response graph.

- The ***Probit Data*** table shows the results of the Probit analysis programme, including χ^2 values, LC values together with the 95% confidence lower and upper limit values, and a variety of other parameters concerning a particular experiment. See Fig. 8.

- The ***TSK*** table shows the results of the Trimmed Spearman-Karber method, including LC50 values with the 95% confidence lower and upper limit values, percent of a Spearman-Karber trim, and a variety of other parameters concerning a particular experiment.

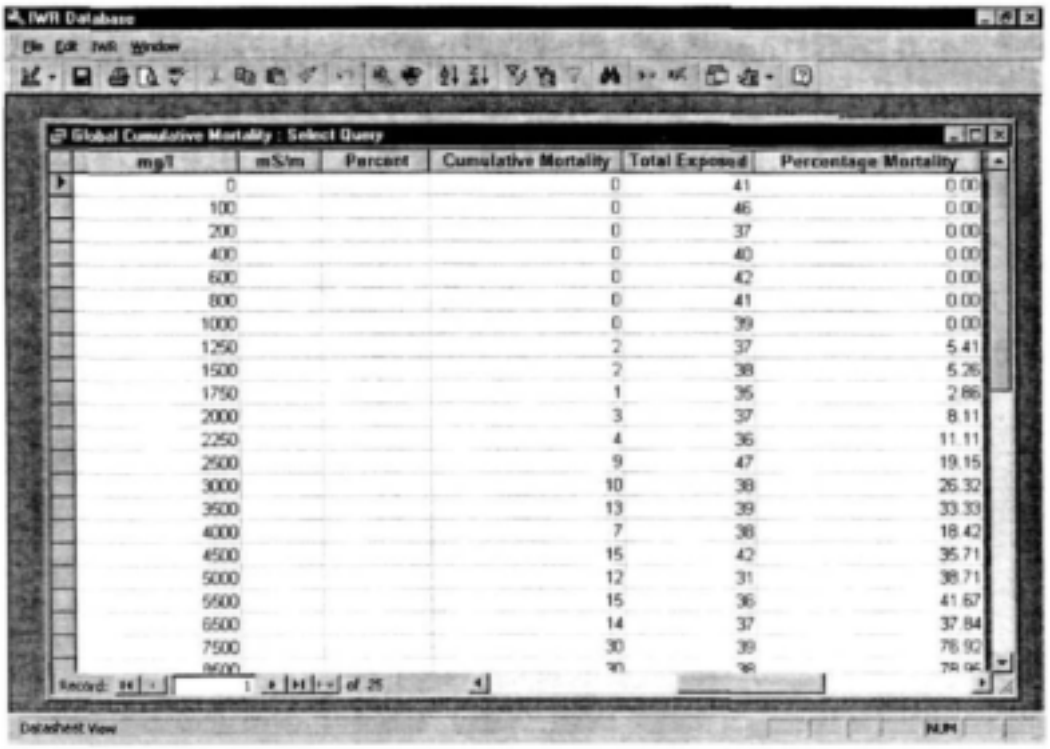

Figure 7
The Cumulative Mortality table from the Report Form.

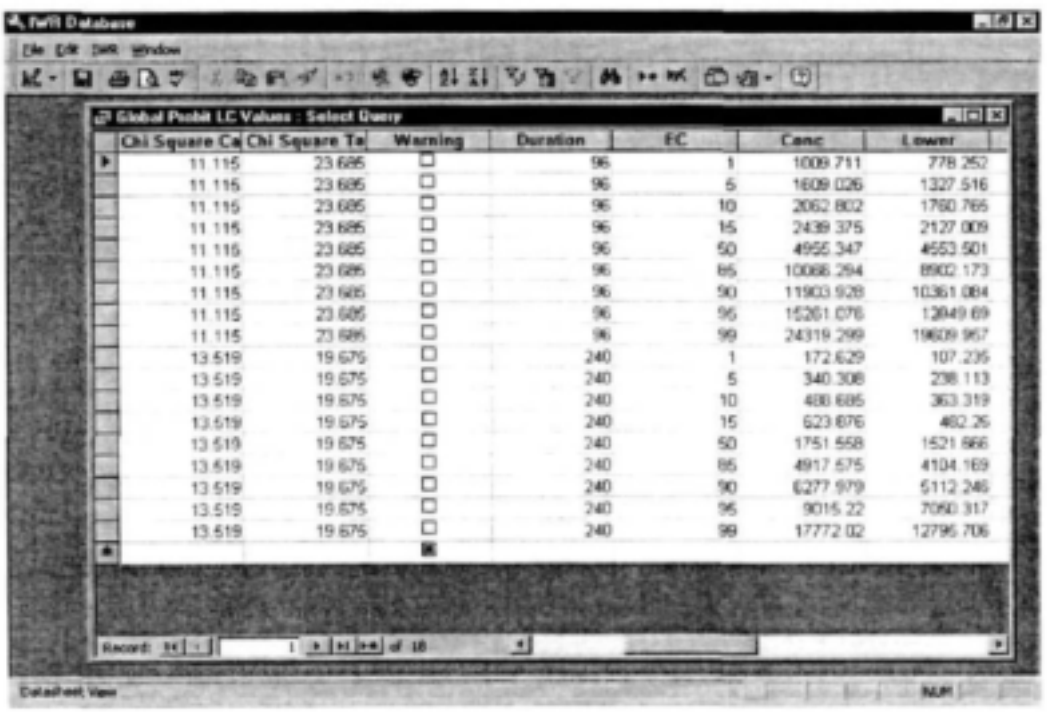

Figure 8
The Probit Data table from the Report Form.

All *query tables* are linked to MS Word and Excel programmes via the ***Office Links*** button. Three options are available for data manipulation: ***Publish it with MS Word***, ***Analyze it with MS Excel*** and ***Merge it with MS Word***.

1.2.5 Database development and quality control

Some of the entry forms for the database are still under development, including:

- ***Sub-lethal end-points***

  As mentioned above, at this stage the database has facilities to accommodate only mortality data as an end-point. It was a first step in the database development to include a facility for storing mortality data, as a large part of the data accumulated by CAT-IWR represents mortality data. Entry forms for sub-lethal end-points should be developed.

- ***ANOVA data***

  As some of the toxicity experiments have been designed to use the ANOVA method for statistical analysis, a special screen to record ANOVA data will be designed. At this stage the database has a screen similar to *Notes* where ANOVA data could be recorded.

To avoid potential problems of uncontrolled access to the data, which could lead to the loss of information, it was decided that only selected staff members of CAT-IWR would have the right to enter information into the database. However, the database would be accessible to a wide range of users. All entries would be checked by the database manager. Mrs L Pakhomova is the CAT-IWR database manager and should be contacted for any queries regarding the database.

APPENDIX 2

SALINITY TOLERANCE DATA

2.1 DATA EXTRACTED FROM THE CAT-IWR DATABASE TO ASSESS THE VARIABILITY OF RESPONSES FROM TOXICITY TESTING USING INDIGENOUS RIVERINE ORGANISMS

To test the variability parameters listed in Section 2.1.1, the following extractions were conducted (Table 1). Probit and Spearman-Karber data was extracted with the selected parameters. For $CaSO_4$ and $MgSO_4$ experiments, only cumulative mortality data was available as data was not appropriate for Probit and Spearman-Karber tests. Due to the quality of this data, these salts were not included in the assessment.

TABLE 1
DATA EXTRACTED FROM THE CAT-IWR DATABASE TO ASSESS DATA VARIABILITY

Parameter	Search term + abbreviated information	Specific data
Test organism	Ephemeroptera + toxicant: Determining variability between mayfly genera, per salt.	1. *Adenophlebia* + NaCl
		2. *Afronurus* + NaCl
		3. *Tricorythus* + NaCl
		4. *Euthraulus* + NaCl
		5. *Adenophlebia* + Na_2SO_4
		6. *Afroptilum* + Na_2SO_4
		7. *Tricorythus* + Na_2SO_4
	Genus + salt: Determining species variability within one genus, per salt.	1. *Tricorythus tinctus* + NaCl
		2. *Tricorythus discolor* + NaCl
		3. *Afronurus peringueyi* + NaCl *
		4. *Afronurus barnardi* + NaCl
		5. *Tricorythus tinctus* + Na_2SO_4
		6. *Tricorythus discolor* + Na_2SO_4
	Ephemeroptera vs. *Daphnia pulex* + NaCl: Determining variability between mayfly data and that of *Daphnia pulex*, using NaCl as toxicant.	1. Mean of mayfly data (if acceptable) + NaCl
		2. *Daphnia pulex* + NaCl
	Ephemeroptera vs. *Caridina nilotica* + Na_2SO_4: Determining variability between mayfly data and that of *Caridina nilotica*, using Na_2SO_4 as toxicant.	1. Mean of mayfly data + Na_2SO_4
		2. *Caridina nilotica* + Na_2SO_4

TABLE 1
DATA EXTRACTED FROM THE CAT-IWR DATABASE TO ASSESS DATA VARIABILITY

Parameter	Search term + abbreviated information	Specific data
Area and river of origin of test organisms	River + genus + salt: Does the river of origin influence toxicity results within a genus, per salt?	1. Sabie River + *Tricorythus* + NaCl
		2. Kat River + *Tricorythus* + NaCl
		3. Breede River + *Tricorythus* + NaCl
		4. Kat River + *Euthraulus* + NaCl
		5. Keurbooms River + *Euthraulus* + NaCl
		6. Vaal River + *Euthraulus* + NaCl
		7. Palmiet River (E. Cape) + *Adenophlebia* + NaCl
		8. Kat River + *Adenophlebia* + NaCl
		9. Vaal River + *Afronurus* + NaCl
		10. Molenaars River + *Afronurus* + NaCl
		11. Sabie River + *Tricorythus* + Na_2SO_4
		12. Olifants River + *Tricorythus* + Na_2SO_4
Toxicant	Organism + NaCl vs. Na_2SO_4: To assess the contribution of the specific salt used as toxicant, to data variability. River of origin, stream type and diluent must be the same for the data being compared.	1. *Tricorythus tinctus* + NaCl - Sabie River, channels + river water.
		2. *Tricorythus tinctus* + Na_2SO_4 - Sabie River, channels + river water.
		3. *Adenophlebia auriculata* + NaCl - Palmiet River, channels + dechlorinated tap water.
		4. *Adenophlebia auriculata* + Na_2SO_4 - Palmiet River, channels + dechlorinated tap water.

TABLE 1
DATA EXTRACTED FROM THE CAT-IWR DATABASE TO ASSESS DATA VARIABILITY

Parameter	Search term + abbreviated information	Specific data
Diluent	Organism + salt (NaCl or Na_2SO) per diluent, i.e. river water vs. dechlorinated tap water or reconstituted water: To test the effect of diluent on the variability of toxicity data. Stream type (+ river of origin, if applicable) must be the same for the data being compared.	1.*Daphnia pulex* + NaCl + river water.
		2. *Daphnia pulex* + NaCl + reconstituted water.
Stream type	Organism + salt (NaCl or Na_2SO_4) per stream type: To test the effect of stream type on the variability of toxicity data. River of origin and diluent must be the same for the data being compared.	1. *Adenophlebia auriculata* + Na_2SO_4 + large artificial stream units (LASU) - Palmiet River, dechlorinated tap water.
		2. *Adenophlebia auriculata* + Na_2SO_4 + raceways - Palmiet River, dechlorinated tap water.
		3. *Adenophlebia auriculata* + Na_2SO_4 + channels - Palmiet River, dechlorinated tap water.

*: Data not used in variability assessment as concentrations tested did not produce a LC50.

APPENDIX 3

TOLERANCES OF SELECTED RIVERINE INDIGENOUS INVERTEBRATES FROM THE SABIE RIVER (MPUMALANGA), AND BUFFALO RIVER (EASTERN CAPE) TO COMPLEX SALINE KRAFT AND TEXTILE EFFLUENTS

Results summarised here are detailed in the WRC report titled "Tolerance of selected riverine indigenous macroinvertebrates from the Sabie River (Mpumalanga) and Buffalo River (Eastern Cape) to complex saline kraft and textile effluents" by Zokufa IS, Scherman P-A and Palmer CG (2001).

3.1 INTRODUCTION

The Department of Water Affairs and Forestry (DWAF) is the custodian of the nation's scarce water resources, and as such, has the task of managing the quantity and the quality of water, thus ensuring sustainability. DWAF has to take active measures to avert or minimize the potential risk of undesirable impacts on the environment. To be able to manage for sustainable use, DWAF has developed a policy of water resource protection (DWAF, 1997). The protection concept is to ensure sufficient water quantity and quality for ecosystem health and to ensure that water resource utilization can be maintained. The implementation of the policy is guided by source-directed controls (SDC) and resource-directed measures (RDM), as regulatory activities. The SDC are aimed at controlling the impacts through use of regulatory measures such as licenses, registration directives and prosecution etc, i.e., setting end-of-pipe effluent standards. The RDM set clear objectives for the desired levels of health for the receiving waters, i.e., instream water quality guidelines (DWAF, 1997).

With the industrialization of society, numerous chemicals are being introduced and used, and many of them end up in the environment. These pose a health hazard to both the environment and the public. Due to these problems, water resources have to be monitored and assessed for the level of impacts. General Effluent Standards and Receiving Water Quality Objectives have failed to improve the quality of water. New Regulations have to be in place to control what is allowed to enter the aquatic environment to protect the environment. DWAF's current legislation, the National Water Act (No. 36 of 1998), ensures the protection of water resources. Generally, wastewaters that enter the aquatic environment are complex mixtures. DWAF has recognised the use of whole effluent toxicity testing (WET) as one of the tools that can be used to minimise impacts in the receiving environment.

Ecological Risk Assessment (ERA) and the toxicity-based hazard assessment are new tools available in South Africa for the protection of water resources, and are used to support sustainable environmental management. The hazard-based guidelines include the concept of "risk to the organisms", and would be used in ERA after the likelihood of exposure to the hazard is determined. Risk, defined as the likelihood that adverse effects will result from exposure (SETAC, 1997), is a fundamental component of DWAF's water resource protection policy (NWA, 1998), and is used in determining effects of impacts on environmental ecosystems. Because of the complexity of ecosystems, the concept of risk incorporates both the variability and uncertainty inherent to biological data (SETAC, 1997).

3.2 AIM

The aim of this study is to investigate the tolerances of selected riverine invertebrates to complex saline effluents from kraft (pulp and paper) and textile processing. Presently, there is very little information on indigenous organism response to saline effluents. This aim has been investigated through a set of six objectives.

3.2.1. Objectives

- To establish a capacity for toxicity testing using flowing water organisms.
- To identify a suitable test organism(s) and to conduct tolerance experiments using these test taxa.
- To contribute to environmental water quality objectives for kraft and textile effluents.
- To assess the toxicity of kraft and textile effluents using WET testing.
- To contribute to the understanding of the effects of kraft and textile effluents on macroinveterbrates in selected rivers.
- To contribute to the toxicity database of the CAT-IWR.

3.3 MATERIALS AND METHODS

In this study, experiments were conducted using a regression design with one channel at each effluent concentration, and a control. A wide range of concentrations was mostly used as they had greater value than few replicated concentrations.

For this study, 2 types of whole effluent were tested:

1. kraft (pulp and paper) effluent
   a) from the mill (excluding bleaching effluent)
   b) irrigation effluent (combined general and bleaching effluents)
2. textile effluent
   a) from the factory (excluding caustic effluent)
   b) post-irrigation (effluent that collected into a holding dam after irrigation).

3.3.1 Collection of effluent, dilution medium and organisms

Grab effluent samples were used during this study as short-term acute toxicity tests were to be conducted, and this method is considered suitable for effluent varying little in composition through time (US EPA, 1992; Burton et al., 1996). Effluent test samples were collected and transported in clean plastic containers to avoid sample contamination. Containers were filled and closed tightly to minimize aeration during transportation, which would result in the loss of volatile chemicals in the effluent and a possible under-estimation of effluent toxicity (APHA, 1992; US EPA, 1992). Preservatives were not added to the samples (Slabbert et al., 1998). The effluent concentration series used in this study was usually 1, 3, 10, 30 and 100% (US EPA, 1985; 1989; 1993). Additional effluent concentrations were used to increase the range. Preliminary testing for groundwater was undertaken to establish its toxicity, as groundwater surfaces as a spring between the irrigated fields and the Elands River (Sabie River study).

Sabie and Buffalo Rivers water, collected from unimpacted flowing water, was used as the dilution medium and control. As the test organisms used in this study are riffle-dwelling mayflies that are in constant contact with flowing water, recirculating systems requiring smaller quantities of dilution medium and generating smaller volumes of wastewater were used. Recirculation also ensured good oxygenation of the water. A one-metre channel was preferred as it promotes rapid and homogenous mixing of the whole

effluent and dilution medium down the channel.

Ephemeropteran mayfly nymphs were collected in fast flowing riffle habitats and used as test organisms. Test organisms were collected by either picking individuals (*Tricorythus tinctus*) off the rocks with a soft paint-brush, as they cling tightly to the rocks, or in the case of the baetid population, by rinsing rocks into a jug of river water, as they easily let loose of their hold on rocks. Baetid population was a complex species and therefore, could not be identified to species levels during collection. The Tricorythidae comprised a single species, *Tricorythus tinctus*.

3.3.2 Experimental procedure

Acute and sub-chronic toxicity tests were conducted using different kraft and textile effluent concentrations. Test organisms were allowed to acclimate to laboratory conditions (Parrish, 1985; APHA, 1992) for 36 hrs before effluent concentrations were added. All toxicity tests included a control to ensure that the responses observed are associated with, or attributed to exposure to the test media, and also to provide a baseline and a point of correction for interpreting the results (Rand et al., 1995). The test channels were placed randomly around the laboratory in an attempt to evenly distribute the variability within the testing environment (Hurlbert, 1984; APHA, 1992; Burton et al., 1996). Mortality, which is defined as immobility of the organism and lack of movement upon touching (Rand and Petrocelli, 1985; APHA, 1992), was used as end-point. Mortality in control greater than 10% was considered unacceptable (Parrish, 1985; APHA, 1992).

Parameters such as electrical conductivity (EC), pH and temperature (per channel and the laboratory) were monitored daily. The values taken at the beginning of the experiment served as a guide to monitor any significant deviation in parameters (every 12 hrs) as the experiment progressed. Mortalities, exuviae and emerged animals were counted, removed and preserved in 80% alcohol. Although moulting, which is the shedding of nymphal skin, is believed to be an environmental response and may be a sensitive indicator of stress in organisms (Diamond et al., 1992), analysis of moulting was not undertaken. Water samples were taken from each channel at the beginning and end of each experiment, preserved with $HgCl_2$, and analysed at the Institute for Water Quality Studies (IWQS).

3.3.3 Data Analysis

Both Probit Analysis and Trimmed Spearman-Karber method were used to determine LC50 values for both kraft and textile effluents. In cases where less than 50% of the population was affected in the highest effluent concentrations, an LC50 value could not be calculated. LC1, LC5 and their 95% confidence limits were generated. Each of these values (or toxicity test end-points) can be associated with a particular hazard description (Palmer and Scherman, 2000). The toxicity test end-points were ranked according to the percentage response, and then related to the resource classification system (Palmer, 1999; Palmer and Scherman, 2000).

Toxicity test results were used to describe site-specific hazard-based guidelines for the effluents tested. This approach, detailed in Palmer and Scherman (2000), should be considered preliminary.

3.4 RESULTS

In this study, attention was paid to the toxicity of two types of kraft effluent: General Kraft Effluent (GKE) and Irrigation Kraft Effluent (IKE); and two types of textile effluent: General Textile Effluent (GTE) and Post-Irrigation Textile effluent (PITE). Ten and seven acute (96 hours) toxicity tests were conducted on kraft and textile effluents respectively; one sub-acute (7 day) on textile, and two short-term chronic (12 day) tests on groundwater.

3.4.1 Water quality

Elands River data was taken from the DWAF (1998) database, for water samples collected from Geluk DWAF sampling site (X2H011Q01). Sabie River water was sampled during 1997 and 1998 toxicity testing experiments. The GKE effluent data were for samples collected from 1997 to 1998, and IKE data from the 1998 samples. Groundwater data were for samples collected in 1998 from a dolomite spring, X-EYE, between the Elands River and the irrigated fields.

Kraft effluent

Salts were a major component of kraft effluent, particularly sodium and sulphate ions. Total Dissolved Solids (TDS) and electrical conductivity (EC) were higher than in either river with sodium, sulphate, chloride and calcium ions being the major contributory factors (Steffen, Robertson and Kirsten Inc., 1990; Dallas and Day, 1993). The Irrigation Kraft Effluent (IKE) showed high levels of chloride (Cl^-), sulphate (SO_4^{2-}), and sodium (Na^+). In some experiments, the pH of General Kraft Effluent (GKE) was higher than 10.0 (alkaline), and the pH of IKE was lower than the GKE indicating the presence of bleaching effluent in the IKE.

Textile effluent

The textile effluent major components were also salts, particularly sodium ions. TDS and EC were about 15 and 20 times higher than the receiving water respectively. The Post Irrigation Textile Effluent (PITE) had higher chloride, calcium and phosphate levels than the General Textile Effluent (GTE). Generally, the nutrient levels were low. Trace metals such as copper (Cu), zinc (Zn) and iron (Fe) were slightly higher than recommended standard limits for irrigation with effluent (DWAF, 1999). This could be expected, as the fabric goes through copper roller printers during printing. The effluent was characterised by a blue-black colour, soapy to touch and smelt of ammonia.

3.4.2 Toxicity results

Table 1 compares the Probit and TSK mortality data of the different kraft and textile

experiments. The groundwater results could not be analyzed using Probit analysis or TSK analysis, as the mortalities were too low. The Probit analysis was chosen as it provided good estimates of the concentrations at which specific mortalities occurred (1% (LC1), 5% (LC5) and 50% (LC50) (with their 95% confidence limits).

TABLE 1

LC50 VALUES AND 95% CONFIDENCE LIMITS, FOR T. TINCTUS,AND BAETIDS IN KRAFT AND TEXTILE EFFLUENTS RESPECTIVELY. RESULTS ANALYSED USING THE PROBIT ANALYSIS PROGRAM VERSION 1.4 AND THE TRIMMED SPEARMAN-KARBER METHOD (HAMILTON ET AL., 1977). (LCL= LOWER CONFIDENCE LIMIT; UCL= UPPER CONFIDENCE LIMIT; χ^2=CHI SQUARE)

Experiment number	Type of Effluent	Probit				Trimmed Spearman-Karber			
		LC50	*95% LCL*	*95% UCL*	χ^2	*LC50*	*95% LCL*	*95% UCL*	*% Trim*
1	General Kraft Effluent (GKE) - 1997	19	16	24	7	20	16	24	0
2		30	26	34	4	30	26	34	24
3		9	2	19	42	14	10	18	0
4		12	8	15	2	11	9	13	0
5	GKE - 1998	38	32	43	1	36	30	42	0
6		64	52	80	7	68	52	87	25
7	Irrigation Kraft Effluent (IKE) - 1998	32	26	36	5	27	22	33	0
8		PROBIT NOT APPROPRIATE				29	25	33	0
9						19	13	28	12
10		32	26	36	5	28	23	32	0
1a	General Textile Effluent (GTE)	25	21	28	7	24	19	29	0
2a		PROBIT NOT APPROPRIATE				7	4	12	28
3a		11	9	13	8	9	8	12	4
4a		6	4	8	8	5	4	7	8
8a		16	14	17	9	14	12	17	0

Toxicity test results using both Probit and Trimmed Spearman-Karber analysis showed little difference in the toxicity of 1998 GKE and IKE samples, with the 1997 GKE samples being more toxic than other samples (Table 1). Therefore, IKE was as potentially toxic to aquatic biota as the GKE during 1998. *T. tinctus* showed sensitivity to kraft

effluent. The textile experiments results indicate that it is not possible to quantitatively compare mayfly responses to both GTE and PITE, but the PITE was clearly more stable and less toxic, with very low responses even at high effluent concentrations. In contrast, the GTE was acutely toxic with the batch used in Experiment 4 being the most toxic (LC50 at 5 - 6% effluent concentration), and the batch used in Experiment 1 the least toxic (LC50 at 24 - 25% effluent concentration). Baetids showed less tolerance to GTE. It is apparent that test responses to each effluent batch was different. The effects varied in different batches, some showing immediate effects, while others showed delayed acute responses.

Figures 1 and 2 show an example of the responses of *T. tinctus* to kraft effluent over 96 hours.

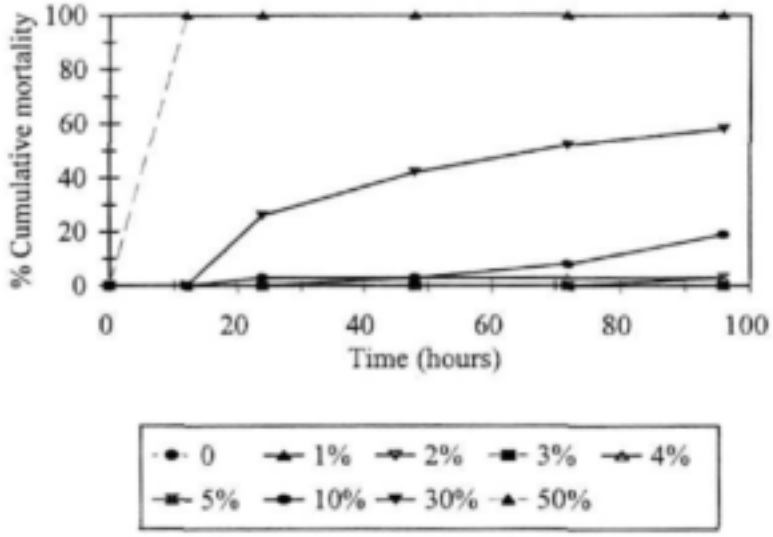

Figure 1

Experiment 1: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to General Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water. The zero mortalities of concentrations in the 0-5% range cannot be distinguished along the x-axis.

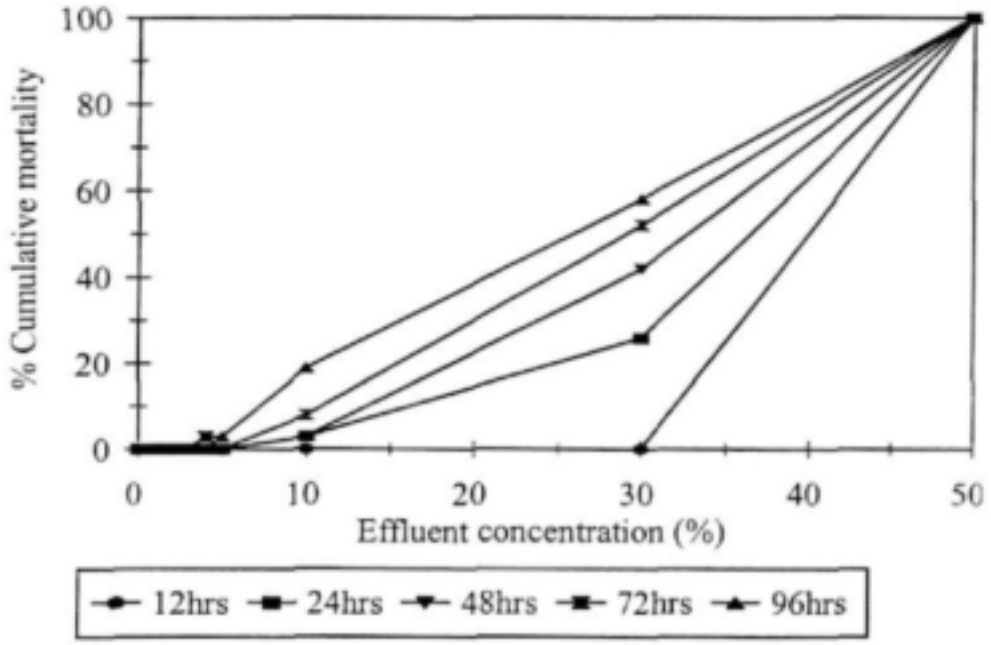

Figure 2
Experiment 1: Concentration-response curve for *T. tinctus* exposed to General Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

3.4.3 Site-specific whole effluent guidelines for kraft and textile effluents

Palmer and Scherman (2000) have developed a method for relating toxicity test data to the resource protection policy of DWAF (Palmer, 1999). This method was applied to each batch of kraft and textile effluents. For each experiment, the tolerance end-points described were LC1, LC5, and LC50 values. Table 2 therefore presents LC1, LC5, LC50 values and their 95% confidence limits, plus the calculated AEV for each GKE, IKE and GTE experiments respectively. The tolerance end-points for each experiment were then ranked and associated with a particular predicted instream river health class. In this study, chronic tolerance tests were not undertaken.

The AEV was calculated in each case according to the method of the DWAF (1996), using LC1 values instead of a mean LC50 value, as a single test species was used to generate toxicity data (Palmer and Scherman, 2000). The AEV value was used, as it indicates the effluent concentration that will cause low risk to intolerant biota, and will therefore give concentration values that will be acceptable for discharge into a class A river. Below the lower 95% lower confidence limit of the LC1 will indicate the threshold of a 95% probability of less than 1% mortality after acute exposure. The LC1 upper confidence limits and LC5 lower confidence limits will indicate moderate risk to intolerant biota, with 95% probability of mortality between 1-5% after acute response. The LC5 is considered high risk and will indicate estimate risk of 5% mortality after acute exposure. The LC1 and LC5 values also have a wide range of confidence limits that will accommodate the nature of biological responses to toxic substances (Rand, 1995).

For each experiment, the tolerance end-points: LC1 and LC5, and the low and upper confidence limits of the LC1 and LC5 values are listed (Palmer and Scherman, 2000).

TABLE 2

LC1, LC5 AND LC50 VALUES OF THE PROBIT ANALYSIS FOR THE INDIVIDUAL KRAFT AND TEXTILE EFFLUENT EXPERIMENTS, THEIR 95% CONFIDENCE LIMITS AND AEV. (UCL = UPPER CONFIDENCE LIMITS, LCL = LOWER CONFIDENCE LIMITS)

Acute (96hr) GKE	LC1	LC1 95% LCL	LC1 95% UCL	LC5	LC5 95% LCL	LC5 95% UCL	LC50	LC95 % LCL	LC50 95% UCL	AEV
Experiment 1	3.8	2.3	5	5.9	4.2	7.7	19.3	16	24	1.9
Experiment 2	4.9	2.3	7.4	8.3	4.8	11.3	30	26.1	35	2.4
Experiment 3	1.1	0	3.6	2.1	0.04	5.4	8.9	2.1	19	0.5
Experiment 4	2.5	0.9	4.2	3.9	1.8	6	11.6	8.2	15	1.2
Experiment 5	15	7.8	19.9	19.4	12	24.5	37.8	32.3	43	7.3
Experiment 6	12	3.9	19.2	19.3	8.8	27.7	63.6	51.9	80.0	5.9
Experiment 7	12.2	6.3	17	16.2	9.7	26.2	31.9	26.2	36.3	6.1
Experiment 10	14.2	7.7	18.9	17.9	11.2	22.5	31.5	26.5	35.3	7.1
Experiment 1a	10.1	6.3	13.2	13.2	9	16.4	25	21.3	28	5.5
Experiment 3a	5.1	2.6	6.9	6.5	3.9	8.2	11.4	9.3	12.9	2.5
Experiment 4a	1.2	0.4	3.1	1.9	0.8	3	6	4.1	7.7	0.6
Experiment 8a	6.3	3.7	8.3	8.2	5.8	10.1	15.6	13.5	17.4	3.1

Each of these values were associated with a particular hazard description, ranked according to the percentage response and then related to the resource classification system. The AEV was used as tolerance end-point to formulate the guidelines. Table 3 presents an example of a ranked list of toxicity test end-points, with a specific hazard description associated with a particular River Health Class, and a resultant hazard-based effluent guideline.

TABLE 3

EXPERIMENT 1, GKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS AND HAZARD-BASED DESCRIPTIONS ARE BASED ON PALMER AND SCHERMAN (2000)

Tolerance test end-points	% effluent concentration	Summarised hazard description	River health class	Suggested % effluent concentration
Chronic test results not available	Unknown	Minimal hazard to intolerant biota – no acute responses	A	0.0 –2.0
AEV	1.9	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	2.0 - 4.0
LC1 lower 95% CL	2.3			
LC1	3.8			
LC5 lower 95% CL	4.2	Moderate hazard to intolerant biota: 95% probability of mortality between 1-5 % after acute exposure	C	4.0 - 5.0
LC1 upper 95% CL	5.0			
LC5	5.9	High hazard: best estimate of 5% mortality after acute exposure.	D	5.0 - 6.0
LC5 upper 95% CL	7.7	Unacceptable hazard: 95% probability of at least 5% mortality after acute exposure	E/F	>6.0

The results show that no more than 2% effluent concentration should be allowed to enter an A Class river, and between 5 and 6% effluent concentration should be the limit in D Class. Once receiving waters have been classified, these results could be used to set appropriate resource quality management objectives. Tables 4 & 5 give a summary of suggested guidelines for the kraft and textile effluents respectively. Table 4 shows the relative similarity of the toxicity, and the required concentration of the 1997 GKE and 1998 GKE and IKE samples. As shown by Table 4, effluent batches for Experiments 1, 2, 3 and 4 appeared to share similar guidelines, and effluent batches in Experiments 5, 6, 7 and 10 also shared similar guidelines. This indicated that effluent of quality similar to batches in Experiments 1, 2, 3 and 4 would have a more serious impact than those with a quality profile similar to the batches of Experiments 5, 6, 7 and 10. The 1998 GKE and IKE samples showed lower toxicity than the 1997 GKE samples.

TABLE 4
IDIVIDUAL KRAFT EFFLUENT EXPERIMENTS WITH ASSOCIATED RIVER HEALTH CLASSES AND ASSIGNED PERCENTAGE EFFLUENT CONCENTRATION GUIDELINE RANGES

River health class	EXPERIMENTS 1, 2, 3 ,4 (GKE 1997) % effluent concentration				EXPERIMENTS 5,6 (GKE 1998) % effluent concentration		EXPERIMENTS 7,10 (IKE 1998) % effluent concentration	
	Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8
A	0 - 2	0 - 2	0 - 0.5	0 - 1	0 - 7	0 - 6	0 - 6	0 - 7
B	2 - 4	2 - 5	0.5 - 1	1 - 3	7 - 15	6 - 12	6 - 12	7 - 14
C	4 - 5	5 - 7	1 - 4	3 - 4	15 - 20	12 - 20	12 - 17	14 - 18
D	5 - 6	7 - 9	4 - 6	4 - 6	20 - 25	20 - 30	17 - 26	18 - 23
E/F	>6	>9	>6	>6	>25	>30	>26	>23

TABLE 5
INDIVIDUAL TEXTILE EFFLUENT EXPERIMENTS WITH ASSOCIATED RIVER HEALTH CLASSES AND ASSIGNED EFFLUENT CONCENTRATION GUIDELINE RANGES

River health class	EXPERIMENT 1 (GTE 1997) % effluent concentration	EXPERIMENTS 3, 4, 8 (GTE 1998) % effluent concentration		
		Exp 3	Exp 4	Exp 8
A	0 - 5	0 - 3	0 - 0.6	0 - 3
B	6 - 10	3 - 5	0.6 - 1	3 - 6
C	10 - 13	5 - 7	1 - 2	6 - 8
D	13 - 16	7 - 8	2 - 3	8 - 10
E/F	>16	>8	>3	>10

From the results presented in Table 5, it could be concluded that any textile effluent similar to the batch for Experiment 3, with effluent concentration greater than 0.6 %, should never enter a Class A river. It also shows that a maximum of 3.0 % effluent concentration will be allowed into a Class D river. Considering the complexity and variability between batches of effluents, and that complex mixtures have different integrated effects on biota, the management should focus on ERA and use it as a tool in its environmental decision-making. The results in Table 4 and 5 indicate that to reduce potential environmental risks, effluent should be diluted before it can be irrigated. These in-stream percentage effluent concentrations could be used to calculate the volume of effluent which should reach the river and groundwater. Although PITE was found not to

be acutely toxic, there is a potential for toxicity. Management should therefore aim for zero effect to protect the environment.

3.4.4 Identification of test organisms

A comparison of baetid population frequencies per experiment, i.e. during the summer of 1997 and 1998, and autumn and winter of 1998 was done. During November 1997 and 1998, *Baetis harrisoni* was in the majority at 95% and *Afroptilum parvum* at 5%. *B. harrisoni* appeared to be a dominant species during summer months (Table 6). This is possibly due to the fact that it is a more tolerant species, and therefore less sensitive (Chutter, 1994). *B. harrisoni* can therefore tolerate the heavy flow and stresses brought about by the environmental changes. During autumn, *B. harrisoni* and *A. parvum* interchangebly shared the dominance. Winter showed *A. parvum* dominating, suggesting that there is not a requirement for a tolerant species to dominate at this time of the year. *Trichorythus* consisted of only one species, *T. tinctus* therefore no identification was necessary.

TABLE 6
COMPARISON OF BAETID POPULATION PERCENTAGE (%) FREQUENCY DURING THE TEXTILE ACUTE AND SUB-CHRONIC TOXICITY TESTING EXPERIMENTS IN 1997 AND 1998

Experiment	*B. harrisoni*	*A. parvum*	Date	Season
1	95	5	24-11-1997	Summer
2	59	41	01-05-1998	Autumn
3	32	68	07-05-1998	Autumn
4	72	38	11-05-1998	Autumn
5	10	90	15-05-1998	Autumn
6	38	72	21-05-1998	Autumn
7	34	67	02-06-1998	Winter
8	95	5	11-11-1998	Summer

3.5 DISCUSSION

WET testing is relatively new in South Africa (Slabbert et al., 1998), particularly using indigenous riverine organisms as test organisms (DWAF, 2000). Currently, the only toxicity database for indigenous invertebrates in South Africa is being developed by CAT-IWR. All data used in the development of SAWQG for aquatic ecosystems (DWAF, 1996) were based on international data. Indigenous organisms were chosen for this study in order to contribute to method development with regard to the use of site-specific macroinvertebrates for regulatory purposes (auditing), and to contribute to the

database for indigenous organisms. The CAT-IWR at Rhodes University in Grahamstown has developed a protocol for acute testing using indigenous organisms in artificial streams, and the information from this study has contributed towards the development of the protocol (DWAF, 2000). This study will particularly contribute to Version 2.0 of the protocol, which will focus specifically on WET testing.

The study aim was to investigate the application of WET testing of complex kraft and textile effluents to assess the potential effects of these effluents on indigenous riverine mayfly nymphs, and to report on the relative toxicity of the recipient groundwater. To achieve this aim, mayfly nymphs (*T. tinctus*) were exposed for 96 hrs to a range of kraft effluent dilutions, and for 12 days to a range of groundwater mixtures. Baetids were also exposed for 96 hrs to a range of textile effluent concentrations. The secondary aim was to provide a set of hazard assessment guidelines, which relate effluent toxicity to river health class. The WET would then be used in testing results in the development of hazard-based guidelines, for the disposal of kraft and textile effluents into the environment. The study represents a first step in developing WET testing methodology using indigenous riverine organisms. WET testing using indigenous riverine organisms can play an important role in auditing permits. Therefore, it is fundamentally important that we have some knowledge of the relationship between the results of laboratory toxicity tests and the actual responses in the receiving water.

3.5.1 The findings of the kraft effluent study

The mayfly nymph *T. tinctus* from the Sabie River in the sub-tropical low veld region of South Africa showed sensitivity to both GKE and IKE. Groundwater also showed some lethal effects to aquatic biota at high effluent concentrations. The species was shown to be highly susceptible to high concentrations of both IKE and GKE. Responses differed from batch to batch of kraft effluents, but generally, *T. tinctus* showed less variability of response to IKE than to the GKE. Interestingly, GKE samples from 1998 were less toxic than GKE tested in winter 1997. The IKEs were more saline than groundwater, which was more saline than the Elands and the Sabie Rivers. Groundwater toxicity was measurable, and therefore groundwater contamination was evident.

The main finding of the study was that kraft effluent is variable and acutely toxic. The toxicity of GKE was more variable than that of IKE, with IKE toxicity close to the mean toxicity of GKE. The IKE collects into a holding dam before it is irrigated, and this could have contributed to its relatively constant toxicity. Groundwater was demonstrably toxic, but over a chronic test period. Although the study cannot demonstrate a *causal* link between groundwater salinisation and toxicity, and the irrigation of toxic effluent, there is a correlation. There is therefore a cause for caution, and this study recommends that IKE should be viewed as having a direct impact on aquatic environments, and should be treated accordingly.

3.5.2 The findings of the textile effluent study

In this study, baetids showed sensitivity to GTE and less sensitivity to PITE. However, since the results are preliminary, a comparative toxicity testing with a *Daphnia* standard laboratory test population, should be undertaken. Other species, such as fish and algae

should also be used as indicated by US EPA (1992), so as to incorporate different trophic levels when generating realistic toxicity data.

In this study, the GTEs were generally found to be variable and acutely toxic, which could be attributed to the fact that there is no secondary treatment, or any form of biological treatment before the effluent is released and used for irrigation. Although PITE was less toxic, this does not mean that it does not impact on the environment. Long-term chronic exposure may show PITE to be toxic. There is a possibility that some substances become trapped in the soil or grass roots during irrigation, as the PITE is a run-off effluent from irrigation. Ions such as Ca^{2+} cations and Cl^- anions are washed down into the holding dam, showing up as high levels of calcium and chloride in the PITE. It has also been shown that Ca^{2+} has an ameliorative effect on toxicity (Palmer and Scherman, 2000). There is also a possibility that non-toxicity is due to the fact that the effluent goes through stabilization period in the holding dam (Tailwater Dam), where some substances such as trace metals attach onto suspended solids and settle at the bottom. Substances such as organics may also be degraded in the Tailwater Dam, rendering the effluent less toxic.

The aim and objectives of this study were met as follows:

- To investigate the effects of kraft and textile effluents to indigenous riverine invertebrates.

Kraft effluent proved to be generally more saline than textile effluent. Textile effluent was more variable than kraft effluent. *T. tinctus* was highly susceptible to high concentrations of both GKE and IKE, and also showed less sensitivity to IKE than GKE. This could be due to the fact that IKE toxicity was close to the mean toxicity of GKE. Baetids were sensitive to GTE, but less sensitive to PITE despite the higher salinity of PITE. This could be due to the ameliorative effects of Ca^{+2} (Palmer and Scherman, 2000), as Ca^{+2} levels in PITE were about double that of GTE. It is not possible to compare the toxicities of the two effluents (i.e. kraft and textile effluents), as different test organisms were used. Sensitivities of the test organisms should however be similar, as they are all Ephemeropteran mayflies.

- To use the tolerance data to derive hazard-based effluent guidelines related to River Health Class.

The results of this preliminary study recommend a 3% effluent concentration guideline for both general kraft effluent (based on mean test results, Table 5) and general textile effluent (based on mean test results, Table 6) for the protection of a Class A river. Ranges for other river classes were also derived. Table 7 lists the recommended guidelines. The LC5 is ecologically more sensible than LC50, as it provides 95% protection for the population. As this study was based on limited data with only one test species or population, the LC1 was used as a conservative estimate of mortality. The LC1 indicates the probability that only 1% of test organisms will disappear from the system. Hazard-based guidelines indicated that low effluent concentrations should enter the receiving water, if the environment is to be adequately protected. Table 7 shows a list of the recommended hazard-based % effluent guidelines for general kraft and textile effluents.

TABLE 7
RECOMMENDED HAZARD-BASED PERCENTAGE EFFLUENT GUIDELINES FOR DISCHARGE OF GENERAL KRAFT AND GENERAL TEXTILE EFFLUENTS. RECOMMENDATIONS ARE BASED ON THE MEANS OF VALUES SHOWN IN TABLES 3.19 AND 4.16

River helath class	Kraft effluent	Textile effluent
A	3	3
B	7	6
C	10	8
D	14	9
E/F	>14	>9

- To identify suitable test organisms for toxicity testing.

Riverine indigenous macroinvertebrates may not be ideal for routine toxicity testing, as there can be no guarantee of their presence in large numbers all year round. However, their use allows a more direct prediction of effects in the receiving environment. For short-term tests, aquatic invertebrates tend to be more tolerant to environmental stressors, and Sloof (1988) warns that the impact of toxicants may therefore be under-estimated. Once methods have been developed, these organisms may be suitable for sub-chronic or chronic toxicity tests, as aquatic organisms tend to exhibit increased sensitivity in long-term tests, possibly due to lower resistance during moulting (Cairns, 1992). A relatively long exposure will probably ensure that most species undergo moulting and that species have complete life cycles during the exposure period. This will however require extensive method development.

This study has shown that untreated kraft pulp and paper mill and textile effluents are acutely toxic, and should not be allowed to enter the receiving environment at any time, as they will have detrimental adverse effects to aquatic biota. To protect the aquatic environment, strict measures should be taken to prevent impacts to water resources. The argument could be that the effluents are not discharged directly to the aquatic environment, but are irrigated. Although effluents are irrigated, the study has shown that kraft effluents impacted on the groundwater, which surfaces as spring-water, and will end up in the river. The impact of textile effluent to groundwater was not investigated, and should be the focus of future research, as preliminary investigations by Bruinette et al. (1997) showed groundwater in the area to be impacted. Effluent monitoring by toxicity testing is recommended for effective environmental management.

The responses of *T. tinctus* confirmed the findings documented in the literature that untreated kraft effluents are toxic. The difference in responses of *T. tinctus* to GKE in 1997 and 1998 could be related to inherent test or experimental variability, or to type of wood species and efficiency in processing (Kovacs and Voss, 1992; Verta et al., 1996).

This is relevant, as GKE is a combination of different streams from the mill. The IKE LC50 values showed similarities between different batches indicating that in the holding dam, there is probably some stabilization of the effluent, thereby reducing the effluent variability.

The percentage frequency of baetids used for textile effluent testing (Table 6), indicated that invertebrate occurrence is related to seasonal effects, e.g. reduction in water volume and flow. Testing during all four seasons would have been ideal, but was not possible as flow rates were drastically reduced during mid-winter and spring months. Collecting organisms from a second site was not considered, in an effort to keep organism genetic variability to a minimum.

The variability of response also reflected the variability from the use of a wild population of indigenous riverine organisms as test organisms. Although the use of indigenous organisms in toxicity tests adds to realism, and the likelihood that results can be successfully extrapolated to site-specific management, one of the major drawbacks is the variability of response in wild populations (Palmer and Scherman, 2000). Toxicity tests were conducted using field-collected organisms, which constitute a wild population. The results of this study were used in the application of a derived hazard assessment guideline, which provides recommended IKE and GTE dilution factors (Table 7), which would render the effluents acceptable in-stream, at different levels of ecosystem health. This information could be used in the decision-making about IKE and GTE treatment and irrigation.

A hazard assessment approach was taken. It was assumed that the statistical information from Probit analysis around acute, lethal responses at low but measurable concentrations could give an indication of the chronic, sub-lethal in-stream biotic response. Further, using the 95% confidence limits around the LC1, and LC5 values allow further quantification of low, but measurable responses, at lower concentrations.

It is an advantage to be able to infer chronic and sub-lethal effect from acute lethal data, since acute toxicity testing is demonstrably cost effective (Cairns, 1983; Rand, 1995). By taking the LC1, LC5, AEV and CEV values as the basis of hazard assessment guidelines for kraft and textile effluents disposal, we are attempting to extrapolate acute sensitivity to the likely biological effect on the environment. We therefore, have linked low levels of response to changes in feeding and breeding, and competitive response would all happen before mortality, as it seems reasonable to infer the possibility of these effects from actual mortality data.

Since both GKE and IKE were found acutely toxic, this study suggests that the mill should focus on ecological risk assessment as a tool for environmental decision-making. It may help the management curb the high cost of eliminating environmental risks associated with effluent impacting on aquatic environment. It is difficult to assess the impact of this whole effluent, as the effluent is not discharged into the river but is used for irrigation. To be conservative, a move toward zero effect would be ideal because of the long-term effect of changes in the groundwater. High levels of sodium and sulphate are of concern, as they can accumulate and affect groundwater. If the groundwater with high sulphate and sodium ions reaches the in-stream environment, it will contribute to

high salinity. Salinity is conservative, and therefore, if resource protection is the goal, attention should paid to the consequences of irrigation.

3.6 CONCLUSION

Whole Effluent Toxicity testing has shown that it can identify responses to complex saline effluents using indigenous riverine invertebrates. WET can also be used by industry to quantify the responses of riverine biota to particular chemical constituents and complex whole effluents. Both *T.tinctus* and baetid test organisms proved to be suitable test organisms for toxicity testing. However, they should be considered as test organisms for auditing purposes, as their availability cannot always be guaranteed. Regular toxicity testing using a standard organism such as *Daphnia* should be included in effluent management programmes. Indigenous riverine invertebrates should be used to set guidelines for ecosystems, as they are representative of the impacted aquatic environment, thereby allowing a more direct prediction of effects.

To more effectively protect the environment, the management structures of both kraft and textile mills should consider adopting Ecological Risk Assessment programmes, as their effluents are toxic and warrants better management. The management would have to be conservative in the manner in which they treat or dispose of their effluent, and should target zero impact. Both effluents should be treated before irrigation to reduce variables that contribute to toxicity. As salinity has been identified as a problem in the Buffalo River (Selkirk and Hart, 1984; DWA, 1986; DWAF, 1991), the discharge of saline effluents into the aquatic environment should be prohibited.

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APPENDIX 4

EFFLUENT STUDIES UNDERTAKEN IN THE VAAL CATCHMENT

Results summarised here are detailed in the WRC report titled "The use of *Daphnia* spp. and indigenous river invertebrates in whole effluent toxicity testing in the Vaal catchment" by Muller WJ and Palmer CG (2002)

4.1 INTRODUCTION

Freshwater is vital to societal, and environmental, well-being and any changes in the distribution, abundance and quality of water resources and ecosystems are detrimental to this societal and environmental sustainability. Increasing socio-economic activities world-wide have been accompanied by increased pollution stress on the aquatic environment. The need for improved efficiency in water quality management is urgent and immediate and it is important that policy to manage freshwater systems is underpinned by sound science. Strategies to manage receiving water quality have been implemented world-wide and include chemical monitoring, biological monitoring and toxicological assessments, all of which are supported by on-going research.

Water quality management in South Africa has come a long way since 1919, when it was first promulgated (Union Health Act 36 of 1919; van der Merwe and Grobler, 1990) but only included sewage effluent. Later amendments broadened water quality management to include effluent discharge from industry, mining and storm-water runoff. However, despite these and uniform, and general effluent standards (UES), as well as DWAFs recognition of the need for integrated water resource management, water quality in the resources continue to deteriorate (DWAF, 1995; Basson et al.,1997).

The National Water Act (NWA) (No 36 of 1998) provides for *resource protection* by the implementation of *resource directed measures* - where the requirements of aquatic ecosystems are provided as quantitative and qualitative resource quality objectives; and through *source directed controls* - where the source of impact (in the case of water quality this means pollutants) is controlled using a licensing system. However, water quality management is particularly complex because it encompasses many different factors and variables - all of which interact.

4.1.1 Whole Effluent Toxicity testing in water quality management

Most pollutants enter water resources (aquatic ecosystems such as rivers, wetlands, dams, lakes, and aquifers) as mixtures. The water in the water resource is also a mixture of water molecules together with a variety of dissolved and suspended substances. These dissolved and suspended substances are termed "pollutants" when they adversely affect people and/or the natural environment.

When a wastewater enters a river or other freshwater system, the wastewater and river water mix and a new, unique chemical mixture forms. Effective water quality management requires that mixtures which threaten people and ecosystems be identified and controlled. Potentially polluting wastewater can enter water resources: directly from industrial outfalls and pipes; together with domestic sewage form the pipes or outfalls from sewage treatment works; or as run-off from the landscape, as non point-source pollution, when water flows over agricultural lands or over formal and informal urban areas.

To date in South Africa, as in many other places, pollution control has mainly been approached by controlling the concentrations of the individual chemical components of mixtures, but this substance-specific, chemical, approach is of limited use in controlling the effects of discharging complex chemical mixtures.

This is because complex chemical mixtures may contain substances that cannot be individually identified; and might be too numerous, too expensive, and/or too difficult, to analyse. Some substances may be present in quantities that cannot be chemically detected, but still have a negative effect. As biological process occur, new mixtures can be formed that are either difficult or impossible to characterise. In addition, mixtures can have substantially different environmental effects than the sum of individual substance effects. Chemical, substance-specific limits, which are dependent on chemical analysis for enforcement, are therefore of limited use in authorising, and controlling the environmental consequences, of the discharge of complex chemical mixtures.

The question is, if chemical testing is inadequate to control the effects of complex mixtures how should they be managed and controlled? The United Kingdom, the United States, and the Netherlands have led the way in introducing direct toxicity assessment or whole effluent toxicity (WET) testing as the way to effectively manage these complex mixtures (Anon, 1989; UK Environment Agency, 1996; Tonkes, 1998; US EPA, 1991a; 2000). This approach involves exposing a variety of test organisms to the complex mixture, and quantifying the effect on test organisms (Grothe et al., 1996). Licence specifications are then in terms of a toxicity test end-point, rather than a chemical concentration. The test organisms respond to the integrated effect of exposure to the whole mixture.

4.2 AIMS

The project aims to investigate and compare the responses of *Daphnia pulex* and site-specific indigenous invertebrates to a range of chemical mixtures in order to evaluate the use of WET testing in water quality management. The site-specific organisms, and some of the wastewaters tested were collected in the Vaal River barrage area, and the results would be particularly applicable in that area.

The specific aims of the project are listed below. During the project, it became obvious that the proposed approach of undertaking acute toxicity tests using selected industry effluents was not feasible as the none of effluents resulted in measurable acute toxic effects. As a result of these practical constraints, the Project Steering Committee agreed that a change in the approach was appropriate and it was decided to create an artificial effluent in order to evaluate *D. pulex* and indigenous invertebrates for whole effluent toxicity testing in the Vaal catchment. Consequently, the proposed aim of relating real industry effluent toxicity test results to industry discharge permits and their actual discharges with a view to reviewing current discharge criteria and receiving water quality objectives set by Rand Water for the Vaal Barrage could not be undertaken. The Steering Committee agreed to the proposed change in application of the toxicity test results listed below (point *iv*).

i.) Identify and access test industries and effluents

- List and visit a range of industries which discharge effluents into the Vaal River (either directly or indirectly via a tributary).
- Select effluents from representative industries in collaboration with Rand Water and DWAF.
- Create an artificial complex effluent mixture.

ii.) Select indigenous invertebrates from a reference site in the Vaal River as test organisms in whole effluent toxicity tests

- Visit a range of possible reference sites identified by previous SASS biomonitoring, and identify one or two abundant indigenous riffle-dwelling (benthic) invertebrate species for use in the artificial streams.
- Set up artificial stream systems at Rand Water Scientific Services.
- Decide on the test medium for the toxicity tests, i.e. river water from the reference site or dechlorinated tap water.
- Undertake preliminary tests to assess the suitability of using the selected test organisms in the artificial streams.

iii.) Use Daphnia pulex and selected indigenous organisms as test organisms in whole effluent toxicity tests

- Undertake acute toxicity tests exposing the standard organism *Daphnia pulex*, with culture medium and river water as dilution waters, to selected industry effluents and an artificial effluent.
- Undertake acute toxicity tests exposing selected indigenous invertebrates, with river water medium as the dilution water, to selected industry effluents and an artificial effluent.

iv.) Relate toxicity test results to the application of WET testing in water quality management

- Evaluate the use of WET testing to the source directed control of complex effluents in the Vaal barrage area.
- Evaluate the use of *D. pulex* and indigenous invertebrates in WET testing.

4.3 BACKGROUND

Whole effluent toxicity (WET) tests are considered useful tools in protecting the environment from the potentially harmful effects of effluents (Chapman, 2000). An underlying assumption of WET tests is that laboratory toxicity tests are predictive of effects in the receiving water resource (Dorn, 1996). Toxicity assessments of effluents is becoming increasingly more common, as it is not possible to test all combinations of the thousands of chemicals in use, for monitoring and regulatory purposes, to try and establish cause-and-effect links (Roux, 1994).

There is sufficient international experience to recommend WET testing as an efficient, and cost-effective approach to the management of complex industrial wastewaters. However, *within* the WET assessment approach, a wide range of tests and test organisms can be used, and it is important that we select the most appropriate combination for South African conditions.

Complex industrial wastewaters can range widely in the threat they pose to human and water resource health. It is therefore important to effectively screen those which pose a low or minimal risk to health, using the shortest and cheapest tests, and then require the lowest level of compliance monitoring. Generally a tiered approach is favoured, where the level of risk posed by the effluent is related to the extent of the testing and monitoring required.

The chemistry of the natural resource into which the effluent flows may mitigate the toxicity of the effluent, and in addition, wild, natural populations may be more tolerant than laboratory-bred test organisms. It may therefore be possible to allow the disposal of effluents that are identified as posing a hazard after testing with standard test organisms. However, as the risk to the environment is increased, the level of knowledge about the effluent; the receiving water; and the response of organisms; that is required increases. Therefore a more relaxed licence condition will carry more onerous testing and monitoring requirements. This is a good example of the "polluter pays" principle.

Within any application of WET testing, the discharger should evaluate the comparative benefits of toxicity identification and reduction compared with the more comprehensive testing required to more accurately assess the risk posed by the effluent. More detailed testing may result in a more relaxed licence criterion, but would also identify unacceptable risks and effluents which should simply not be discharged to the environment without toxicity identification and reduction.

In this study, *D. pulex* were exposed to an artificial effluent first using laboratory medium as the test diluent; then using river water as the diluent - thus taking into account the possible mitigating affect of receiving water chemistry. Then followed tests using indigenous, site-specific riverine organisms as test taxa, which took into account any differences in tolerance between the wild populations from the receiving water resource as compared with a standard test organism.

4.3.1 Whole effluent toxicity (WET) testing and aquatic toxicology

Since a purely chemical analytical approach is unable to protect the aquatic environment, and chemical monitoring does not take into account either environmental factors which affect toxicity (e.g. hardness, pH, temp, suspended inorganics, dissolved organics); or the potential transformations such as speciation which chemicals can undergo and may result in altered toxicity; WET methods are considered suitable for predicting instream effects.

Aquatic organisms integrate the effects of their environment during their lifetime, and can therefore be used in experiments (bioassays) and also to reflect conditions to which they may have been exposed to earlier (biomonitoring). Bioassays, both toxicity tests and bioaccumulation studies, have been shown to be highly effective in water quality monitoring programmes in developed nations, where improvements in environmental water quality have been achieved (Chapman, 1995).

Bioassays, complemented by chemical and biomonitoring data, are essential tools for the assessment of effluents: both biological data and chemical data together are important for the management of the quality of waste-water and the receiving water bodies.

There are numerous ways to assess the toxicity of inputs into the aquatic environment and these are mainly standard acute and chronic tests (bioassays) in order to show either "end-of-pipe" toxicity or receiving water toxicity (Roux, 1994). Acute toxicity tests are generally less than four days, and usually mortality is measured, while chronic toxicity tests assess the effects of long-term exposure to a toxicant, typically expose the test organisms over at least part of their life-cycle and measure responses such as growth and reproduction (Rand, 1995). Acute toxicity tests tend to be cheaper to perform and are performed most commonly.

The specifics (i.e. choice of test organism, exposure system, dilution water) of undertaking WET tests are determined by the fundamental question being addressed; and need to take variability in to account.

4.4 MATERIALS AND METHODS

Laboratory toxicity tests are considered the first step in a tiered approach in establishing acceptable levels of effluents and standard experimental protocols for toxicity tests are accepted as suitable methods in establishing the toxicity of effluents. The standard methods for undertaking acute toxicity tests were for a static test system and a recirculating test system. The static test uses the standard laboratory-reared test organism *D. pulex*, while the recirculating test method uses field-collected, site-specific, indigenous invertebrates. The field-collected organisms used were two species of mayfly, namely *Afronurus peringueyi* (Heptageniidae) and *Euthraulus elegans* (Leptophlebiidae). The site-specific organisms were collected from the Vaal River at Engelbrechtdrift Weir, which is downstream of the Vaal Dam and upstream of the Vaal Barrage. The Vaal Barrage receives input from several tributaries draining the industrial complexes of the Vaal Triangle and Johannesburg; data collected showed that Engelbrechtdrift Weir is significantly less impacted than a site downstream of the Vaal Barrage.

In collaboration with Rand Water, a number of industry effluents from the Vaal Triangle were selected for toxicity testing. However, these effluents proved to have sub-lethal rather than acute effects, and an artificial effluent was created based on results by Slabbert et al. (1998). The artificial effluent was based on a metal plating industry effluent, and was known to have an acutely toxic effect on both *D. pulex* and fish. A stock solution was made at the start of the experimental period, and the same stock was used to make effluent concentration ranges for both *D. pulex* and site-specific indigenous invertebrate experiments.

The experimental design followed was a regression design and, in order to improve the prediction of point estimates (e.g. LC50), the minimum number of effluent concentrations used in the experiments was 9 for the site-specific indigenous invertebrate experiments and 8 for the *D. pulex* experiments. In order to test the proposed tiered approach for WET testing, and increase environmental realism, both receiving water and culture water were used to make effluent concentration ranges. Receiving water was used as dilution water to make effluent concentrations for the recirculating experiments, while both receiving water and culture water were used to make effluent concentrations for the *D. pulex* experiments in order to establish whether dilution water affected toxicity of the artificial effluent.

In order to standardise between the two experimental methods as far as possible, the experimental end-point measurement for all test species was mortality as this is widely accepted to be an unambiguous measure of response. The standard method for the static test was the 48-hour acute toxicity test method as specified by US EPA (1991b) and the standard method for the recirculating test was the 96-hour acute toxicity test method for artificial streams as specified by DWAF (2000). Four experiments were undertaken using the site-specific mayflies and 3 experiments were undertaken using *D. pulex*.

Data were analysed using Probit and trimmed Spearman-Karber; lethal point estimates and confidence intervals were obtained. Various methods (e.g. the APHA formula and coefficient of

variation) were used to assess variability both within species (i.e. between experiments) and between species, and whether differences between LC values were significant.

4.5 RESULTS AND DISCUSSION

Acclimation mortality for the site-specific indigenous invertebrates was low (<3% across all four experiments) and there were no control mortalities. Similarly, there were no control mortalities for the *D. pulex* experiments. Therefore, the toxicity test results for all species were considered acceptable.

Species differ considerably in their sensitivity to toxicants (Table 1) and using site-specific indigenous invertebrates in toxicity tests allows for a more accurate prediction of the effects of pollutants in receiving water. *D. pulex* in culture water had the lowest LC50 value (0.2-0.4% effluent), which was significantly lower than the LC50 value for *D. pulex* tested in receiving water (0.4-1.3% effluent) and the LC50s for *D. pulex* was significantly lower than for both the site-specific indigenous invertebrate species tested. There was a significant difference in sensitivity between the two site-specific invertebrates tested, with the leptophlebid (*E. elegans*) being significantly more sensitive than the heptagenid (*A. peringueyi*) (LC50s were 8.9-12% effluent and 40-214% effluent, respectively).

The coefficient of variation (%CV) between experiments was lowest for *E. elegans* (18%) suggesting that between experiment variability for this species was acceptable (there was also no statistically significant difference in point estimates between experiments). The %CV for *A. peringueyi* was considered too high (76%) (although no statistically significant difference could be established between the experiments), but this may have been a result of the organisms tolerance to the artificial effluent: 100% cumulative mortality was not reached in any of the experiments and the confidence intervals for the predicted point estimates were wide. Similarly, although due to their sensitivity rather than their tolerance, it was difficult to find an appropriate concentration-range for *D. pulex* and %CVs are high. The confidence intervals for *D. pulex* tested in receiving water are wider (53%) than those for *D. pulex* tested in culture water (49%), which concurs with the general view that increasing environmental realism (i.e. use of receiving water) results in increased variability in experimental results but this increased variability is no greater than variability experienced in effluent toxicity tests. There was no more variability in the toxicity test results (as assessed by %CV) as a result of the selected test system, i.e. static or recirculating test system. This suggests that the recirculating test system using artificial streams is suitable for assessing the toxicity of effluents in the Vaal River catchment.

The intention of effluent testing programmes world-wide has been to reduce the toxic effects of discharges of receiving water resources and WET testing has been shown to be effective in achieving the aim of reducing the discharge of toxic discharges from point sources although there are a number of sources of variability which may affect the interpretation of toxicity test results. Variability is an inherent part of effluent toxicity tests, and it has been suggested that rather than attempting to reduce the sources of variability, by e.g. developing further new acute toxicity tests, the sources of variability should be acknowledged, and incorporated, in the interpretation of the test results.

The results presented in this pilot study are for acute toxicity tests using an artificial metal plating industry effluent. They provide good evidence that the tiered approach for the assessment and

effective management of complex waste discharge, in this case metal plating industry effluent, to the Vaal River catchment may be useful and appropriate. The first level of testing would require a standard laboratory-reared organism, such *D. pulex*, to be tested in culture water; this is the cheapest test method, but would also be the most protective for the receiving environment (i.e. most stringent criteria). The next tier of testing would increase environmental realism, by introducing use of receiving water in toxicity tests. This increases variability of the toxicity test result, which may make the test result more complex to interpret, but may provide more relaxed criteria for discharge by industry. The third level, which is more expensive, but increases environmental realism further through use of site-specific indigenous invertebrates may show that less stringent criteria may be applied and still be protective of the receiving water.

However, it is important to note that acute toxicity tests are short-term tests (48-hours for the *D. pulex* and 96-hours for the site-specific indigenous invertebrates), represent an extreme response (mortality) and the effluent used in the toxicity tests was an artificial effluent. The data provided therefore only provide evidence that the selected management approach is appropriate for acutely toxic effluent, where the end-point measurement is mortality. Numerous experiments undertaken over a period of two years, using a range of industry effluents from the Vaal Triangle, indicated more subtle and sub-lethal effects on the selected test organisms (these data will be archived in the toxicology database currently housed at CAT-IWR). This has implications for the effective management of water quality of the receiving water resource, the Vaal River.

The fact that no acutely toxic effluent was found during the experimental periods does not mean that no acutely toxic effluent is being discharged to the Vaal River. It rather indicates that there are numerous discharges to the receiving water which have sublethal and chronic effects which will not be adequately assessed during short-term (acute) toxicity tests. Short-term toxicity tests do not provide accurate predictions of longer-term environmental effects and it is imperative that more ecologically relevant toxicological data be generated in order to more accurately predict environmental effects of effluents.

Therefore, in order to effectively manage the water quality of the Vaal River catchment, it is important that sublethal and chronic toxicity testing be undertaken so that all the effluents can be adequately assessed.

Data and experience obtained through the course of this research project has highlighted gaps and shortcomings in the current toxicological knowledge-base in South Africa. A number of recommendations for immediate future aquatic toxicology research in order to address these and obtain useful toxicological data and understand how best to apply the data for effective management of South Africa's water resources are listed:

i.) Continue to undertake acute toxicity tests using (site-specific) indigenous test organisms, and using both single-substances and whole effluents, in order to better understand variability in toxicity data associated with using different organisms, different chemicals and different site-specific receiving waters.

ii.) There is an urgent need for the development of chronic and sub-lethal test methods, and appropriate test end-point measurements, using indigenous organisms.

iii.) Develop methods which can link laboratory toxicity test results, both chronic and acute, to instream biological assessments.

iv.) Development of new toxicity test methods to assess effects of pulsed exposure to chemicals, undertake *in situ* toxicity assessments and assess sediment toxicity.

TABLE 1

LETHAL END-POINT PREDICTIONS (LC1, LC5, LC10 AND LC50) (95% CONFIDENCE INTERVALS) AND TOXIC UNITS (TU) FOR THE ARTIFICIAL METAL-PLATING INDUSTRY EFFLUENT (% EFFLUENT CONCENTRATION) FOR *AFRONURUS PERINGUEYI* (HEPTAGENIIDAE), *EUTHRAULUS ELEGANS* (LEPTOPHLEBIIDAE) AND *DAPHNIA PULEX* (DAPHNIIDAE). WHERE THE PROBIT MODEL DID NOT FIT THE DATA, TRIMMED SPEARMAN-KARBER LC50 RESULTS ARE PRESENTED AND NO FURTHER POINT-ESTIMATES ARE LISTED

Experiment	LC1	LC5	LC10	LC50	Model	TU
A. peringueyi						
1	-	-	-	40 (26-61)	TSK (30%)[1]	2.5
1-trimmed	1.3 (0.4-2.6)	3.8 (1.8-6.2)	6.7 (3.7-10)	49 (34-79)	Probit	2.04
2	4.9 (0.8-10)	14.7 (5.6-24)	27 (14-41)	214 (112-959)	Probit	0.47
3	7.9 (2.8-13)	15 (7.6-22)	22 (13-30)	76 (56-120)	Probit	1.31
4	3.7 (0.7-7.9)	11 (4.3-19)	21 (11-32)	171 (94-604)	Probit	0.59
%CV	26	3	12	76		
E. elegans						
1	-	-	-	8.9 (7.6-11)	TSK (3.6)	11.2
1-trimmed	-	-	-	9.1 (7.6-11)	TSK (3.6)	10.9
2	1.7 (0.9-2.5)	2.9 (1.8-4.1)	3.9 (2.6-5.3)	12 (9.4-15)	Probit	8.3
3	-	-	-	8.9 (7.1-11)	TSK (12%)	11.2
4	-	-	-	11 (9.1-14)	TSK (10%)	9.1
%CV	-	-	-	18		

TABLE 1

LETHAL END-POINT PREDICTIONS (LC1, LC5, LC10 AND LC50) (95% CONFIDENCE INTERVALS) AND TOXIC UNITS (TU) FOR THE ARTIFICIAL METAL-PLATING INDUSTRY EFFLUENT (% EFFLUENT CONCENTRATION) FOR *AFRONURUS PERINGUEYI* (HEPTAGENIIDAE), *EUTHRAULUS ELEGANS* (LEPTOPHLEBIIDAE) AND *DAPHNIA PULEX* (DAPHNIIDAE). WHERE THE PROBIT MODEL DID NOT FIT THE DATA, TRIMMED SPEARMAN-KARBER LC50 RESULTS ARE PRESENTED AND NO FURTHER POINT-ESTIMATES ARE LISTED

Experiment	LC1	LC5	LC10	LC50	Model	TU
***D. pulex* in culture medium dilution water**						
1	0.11 (0.03-0.2)	0.17 (0.07-0.2)	0.2 (0.09-0.3)	0.4 (0.3-0.5)	Probit	250
2	0.05 (0.02-0.07)	0.07 (0.04-0.1)	0.09 (0.06-0.1)	0.2 (0.15-0.3)	Probit	500
3	-	-	-	0.17 (0.15-0.18)	TSK (0%)	588
%CV	57	54	52	49		
***D. pulex* in Vaal Dam dilution water**						
1	-	-	-	0.4 (could not be estimated)	TSK (25%)	250
2	-	-	-	0.9 (0.6-1.4)	TSK (2.5%)	111
3	-	-	-	1.3 (1-1.7)	TSK (0%).	77
%CV				53		

[1] Denotes the % Trim for the Trimmed Spearman-Karber analysis.

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Standard laboratory organisms for water quality studies programme

EH Haigh, H Davies-Coleman

This project is part of a thrust to develop methods of ecotoxicological testing using indigenous organisms from flowing waters. The aim of this particular project was to screen riverine organisms for species suitable for laboratory maintenance, develop a pilot programme for the maintenance of laboratory populations, and to establish a supply of suitable taxa for experimental purposes. The criteria on which the organisms were selected include their sensitivity, aspects of their ecology and life history, physiology and the ease with which they can be cultured and handled. The organism most successfully cultured was the freshwater limpet *Burnupia stenochorias*. The mayflies *Adenophlebia auriculata* and *Choroterpes elegans* were also reared successfully in the laboratory, but have not yet been bred successfully artificially.

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Development of a production facility for test organisms to be used in flowing water ecotoxicological research

EH Haigh, H Davies-Coleman

The WRC has supported a suite of projects investigating the tolerance of indigenous organisms to variations in water quality. One of the constraints identified is availability of standard organisms of known age and origin for use in ecotoxicological tests. Without this, the variability of the results obtained is likely to be increased by an unknown amount. This is the second project in a series which investigated the possibility of rearing suitable animals in the laboratory for use in water quality testing. The first project screened a series of organisms and this project has enabled the researchers to investigate two candidate species in some depth. The two candidate species are the freshwater limpet *Burnupia stenochorias* and the mayfly *Adenophlebia auriculata*. Another aim of the project was the design of a facility dedicated to rearing invertebrates of use in ecotoxicological work.

Further fieldwork was conducted on both the organisms. In the laboratory, progress has been made with the culture of *B. stenochorias*, particularly in the realms of feeding, growth rate and fecundity determinations. Handling of the limpets, which had proved difficult, was overcome successfully. *A. auriculata* proved easy to rear in the laboratory, but the constraint is in the breeding. Some success was achieved in the artificial fertilisation of the eggs, but these did not hatch. However, the organism is readily available in the field and so field-caught nymphs may be used experimentally.

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