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

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ABSTRACT

Whole Effluent Toxicity (WET) testing has been identified as one of the tools in the management of complex effluents in aquatic ecosystems. In South Africa, toxicity testing has not been required for regulatory purposes. Recently, the Department of Water Affairs and Forestry has adopted WET testing as a tool to evaluate the suitability of hazardous effluent for discharge into receiving environments. This has necessitated suitable procedures to be established for use in the South African situation. With the implementation of the new National Water Act (No 36 of 1998), industries have to comply with set standards to protect the aquatic environment. However, the South African Water Quality Guidelines for Aquatic Ecosystems have been set using international toxicity data, and it is not known if they are comparable with South African conditions.

The aim of this study was to investigate the tolerances of selected indigenous riverine invertebrates to complex saline effluents. The study investigated the effects of kraft mill effluent to *Tricorythus tinctus*, a tricorythid mayfly from the Sabie River, Mpumalanga, and the effects of a textile effluent to baetid mayflies of the Buffalo River, Eastern Cape. Indigenous riverine invertebrates were chosen as test organisms, as there is no toxicity data in South Africa which could be used to evaluate the level of protection afforded by the South African Water Quality Guidelines for Aquatic Ecosystems. The use of indigenous riverine invertebrates added the challenge of variability of a wild population, and the use of a complex effluent as toxicant added the variability of effluent composition.

In this study, WET testing was used to determine the dilution of whole effluents required for discharge. Hazard-based guidelines were developed for the disposal of kraft and textile effluents. The level of environmental hazard posed by different effluent concentrations was ranked, and was related to the River Health Class. This indicated effluent concentrations that may be allowed to enter the aquatic environment, e.g. 3% effluent concentration guideline for both general kraft effluent and general textile effluent for the protection of a Class A river. This approach could contribute to the use of an Environmental Risk Assessment, approach for the

management of complex effluents.

A number of acute 96 hour toxicity tests were conducted following an unreplicated regression design, using kraft and textile effluents as toxicants, mayfly nymphs as test organisms, and river water as diluent and control. Test organisms were sampled from unimpacted, flowing-water riffle areas, and were exposed in recirculating artificial streams (or channels) to a range of effluent concentrations. Mortality was selected as end-point and observed twice daily.

The experimental results showed the variability and acute toxicity of both kraft and textile mill effluents. Baetids were more sensitive (mean LC50=16% effluent concentration) to General Textile Effluent (GTE), but less sensitive to Post Irrigation Textile Effluent (PITE). Textile effluent (PITE) held in a holding dam were therefore less variable and less toxic; suggesting that stabilization of the effluent could have contributed to reduced toxicity. Effluent composition, e.g. higher calcium levels, may also have contributed to lowering toxicity. *T. tinctus* was sensitive to kraft effluents, but showed less variable responses to Irrigation Kraft Effluent than General Kraft Effluent.

Toxicity test data indicated that GKE, IKE and GTE should not enter the aquatic environment without treatment, as they can cause adverse effects to aquatic biota. Both kraft and textile effluents must therefore be treated before discharge. Different responses to different effluent batches were probably due to effluent variability. The use of indigenous organisms, and not a standard laboratory organism, could also have contributed to variability. A hazard-based approach could be useful, as it will provide a consistent basis for deciding on the acceptability of impacts, while allowing natural site-specific differences to be taken into account.

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GLOSSARY

The definitions have been extracted from Rand (1995), unless otherwise stated.

Acclimation is the time period prior to the initiation of a toxicity test in which aquatic organisms are maintained in untreated, toxicant-free dilution water.

Acute Effect Value is the concentration at and above which a statistically significant acute adverse effect is expected to occur (Roux *et al.*, 1996).

Additive toxicity is the toxicity of a mixture of chemicals that is approximately equivalent to that expected from a simple summation of the known toxicities of the individual chemicals present in the mixture.

Antagonism is a phenomenon in which the toxicity of a mixture of chemicals is less than that which would be expected from a simple summation of the toxicities of the individual chemicals present in the mixture.

Aquatic toxicology is the study of the effects of manufactured chemicals and other anthropogenic and natural materials and activities (collectively termed toxic agents or substances) on aquatic organisms at various levels of organization, from sub-cellular through individual organisms to communities and ecosystems.

Assimilative is the ability of the receiving water to dilute or degrade the **capacity** pollutant without damage to the aquatic environment (Grothe *et al.*, 1996).

Bioaccumulation is a process by which chemicals are taken up by aquatic organisms directly from aquatic environment as well as through exposure from other routes.

Bioassay is a test used to evaluate the relative potency of a chemical or a mixture of chemicals by comparing its effect on a living organism with the effect of a standard preparation on the same type of organism.

Bioavailability is the portion of the total quantity or concentration of a chemical in the environment, or a portion of it that is potentially available for biological action, such as uptake by an aquatic organism.

Biodegradation is the transformation of a material resulting from the complex enzymatic action of microorganisms.

Biological Oxygen Demand is the amount of dissolved oxygen consumed by organisms in water rich in organic matter (DWAF, 1996a-f).

Biomonitoring	is the use of living organisms as indicators in water quality surveillance and compliance to detect changes in effluent and water bodies and to indicate whether aquatic life may be endangered.
Chemical Oxygen Demand	is the amount of oxygen required to oxidize all the organic matter that is susceptible to oxidation by a strong chemical oxidant (DWAF, 1996a-f).
Chronic Effect Value	is the concentration limit, which is safe for all or most of populations even during continuous exposure (Roux *et al*., 1996).
Concentration-response curve	is a curve describing the relationship between different exposure concentrations of the test material and percentages response of the exposed test population.
Control	is a treatment in a toxicity test that contains no toxicant.
Dilution water (diluent)	is the water used to dilute the test material in an aquatic toxicity test in order to prepare different concentrations of an effluent for the various test treatments.
Ecological Reserve	is the quantity and quality of water required to protect aquatic ecosystems in order to secure ecologically sustainable development and use of relevant water resource (DWAF, 1998).
Ecological Risk Assessment	is the process of estimating and characterizing the likelihood that adverse effects of human actions on the non-human environment will occur, are occurring, or have occurred.
End-point	is the adverse biological response that is measured and used as criteria for effects.
Hazard	is the potential to have an adverse effect.
Mayflies	are a group of insects with aquatic nymphs, generally sensitive to polluted conditions e.g. Order Ephemeroptera, families Tricorythidae, Baetidae and Leptophlebiidae.
Median effective concentration	is the concentration of material in water to which test organisms are exposed that is estimated to be effective in producing some sub-lethal response in 50% of the test organisms.
Median lethal	is the concentration of material in water to which test organisms

concentration	are exposed that is estimated to be lethal to 50% of the test organisms.
Mercerizing	is the treatment of cotton fabric with concentrated sodium hydroxide to impart sheen and improve the wettability of the fabric (Correia *et al*., 1994)
Monotonicity	is when the response proportions consistently increasing with increasing concentrations, is not maintained.
Pseudo-replication	is the use of inferential statistics to test for treatment effects with data from experiments where either treatment is not replicated, or replicates are not statistically independent.
Reference site	is a relatively un-impacted site used as a basis for comparison with the sampling site.
Reserve	is the quantity, quality and reliability of water needed to protect both basic human needs, and the structure and function of ecosystems so as to secure ecologically sustainable development and utilization (DWAF, 1997c).
Resource-directed measures	are measures to control the quality of effluents discharged into water resources, to ensure that it is protected (DWAF, 1997b).
Risk	is the likelihood that adverse effects will result from exposure (SETAC, 1999).
Single-species test	is a test where only one species is used as test organisms.
Standard laboratory (test) organism	is an organism that has been bred and reared in the laboratory for the purpose of being used in toxicity testing.
Source directed controls	are measures used to prevent or minimize wastewater discharges that might impact on the aquatic environment (DWAF, 1997a).
Synergism	is a phenomenon in which the toxicity of a mixture of chemicals is greater than that which would be expected from a simple summation of the toxicities of the individual chemicals present in the mixture.
Target Water Quality Range	is a management objective used to specify the desired concentration range and water quality requirements for a particular constituent (DWAF, 1996f).

Toxicant	is an agent or material capable of producing an adverse response (effect) in a biological system, seriously injuring structure and/or producing death.
Toxicity test	is the means by which the toxicity of a chemical or other test material is determined.
Water quality criteria for aquatic ecosystem	are numerical values or narrative statements that are calculated from experimental data and based on expert opinion, with the aim of protecting the aquatic environment.
Water quality guideline	is 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.
Whole effluent toxicity	is the total effect of an effluent measured directly with organisms in a toxicity test.

ACRONYMS

AEV	Acute Effect Value
ANOVA	Analysis of Variance
APHA	American Public Health Association
ASTM	American Society for Testing Material
BATNEEC	Best Available Technology Not Entailing Excessive Cost
BOD	Biological Oxygen Demand
CAT-IWR	Centre for Aquatic Toxicology- Institute for Water Research
CEV	Chronic Effect Value
COD	Chemical Oxygen Demand
DWAF	Department of Water Affairs and Forestry
EC	Electrical Conductivity
EC50	Median Effective Concentration
EDTA	Ethylenediaminetetraacetic acid
EMC	Ecological Management Class
ERA	Environmental Risk Assessment
FAV	Final Acute Value
GKE	General Kraft Effluent
GTE	General Textile Effluent
IKE	Irrigation Kraft Effluent
ISO	International Organization of Standards
IWR	Institute for Water Research
IWQS	Institute for Water Quality Studies
LC50	Median lethal concentration
NOEC	No observed effect concentration
NRHP	National River Health Program
OECD	Organization for Economic Co-operation and Development
PITE	Post-Irrigation Textile Effluent
RDM	Resource-directed measures

RWQO	Receiving Water Quality Objectives
SASS	South African Scoring System
SAWQG	South African Water Quality Guidelines
SDC	Source-directed controls
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
TWQR	Target Water Quality Range
UES	Uniform Effluent Standards
USEPA	United States Environmental Protection Agency
WET	Whole Effluent Toxicity

CHAPTER 1

TOXICOLOGY AND WATER QUALITY MANAGEMENT IN SOUTH AFRICA

1.1 INTRODUCTION

South Africa is an arid or semi-arid country with a rainfall that is highly seasonal, and unevenly distributed across the country. The mean annual rainfall (MAR) is about 500mm, and is below the world average of 860mm. Approximately 65% of the country receives less than this annually, and 21% of the country receives less than 200mm (DWA, 1986). In many rivers flow is seasonal, creating variable flow rates. The impoundment of rivers and abstraction of water for water supply purposes severely affects the natural flow of many rivers. Changes in flow conditions may cause stress to aquatic ecosystems from habitat loss, as well as from changes in water quality because of a reduction in dilution capacity. High evaporation rates reduce the availability of surface run-off from rainfall and cause losses from water stored in dams (DWA, 1986). The available water resources are over-utilised and many areas of South Africa experience water stress due to over-allocation, competing water use, variability in in-stream flows etc.

The Department of Water Affairs and Forestry (DWAF) is the custodian of the nation's water resources, and as such has the task of managing the quantity and the quality of water. DWAF is responsible for ensuring that South Africa's water resources remain "fit for use" on a sustainable basis, and also has to take active measures to avert or minimise the potential risk of undesirable impacts on the environment (DWAF, 1996a-f). To be able to manage for sustainable use, DWAF has developed a policy of water resource protection (DWAF, 1997a). The South African Water Quality Guidelines for Aquatic Ecosystems (DWAF, 1996f) are used in the implementation of this policy. The protection concept is to ensure sufficient water quantity and quality for ecosystem health and to ensure that water resource utilisation can be maintained. The implementation of the policy is guided by source-directed

controls and resource-directed measures, as regulatory activities. The **source-directed controls** are aimed at controlling impacts through the use of measures such as licenses, registration directives and regulations, such as setting end-of-pipe wastewater standards. **Resource-directed measures** set objectives for the desired status of water resources, i.e. in-stream water quality guidelines (DWAF, 1997a).

The DWAF's current legislation, the National Water Act (No. 36 of 1998), establishes the protection of water resources as a right in law. According to Section 18 of the Act, the Minister must give effect to the Reserve, which is defined as the quantity and quality of water required to satisfy basic human needs and to protect aquatic ecosystems (NWA**,** 1998). There is the basic human needs Reserve and the ecological Reserve. The ecological Reserve consists of environmental requirements for water quality and quantity. The DWAF policy directs that treated sewage and industrial effluents be returned to the water bodies, from which the water was originally abstracted, thus supplementing the declining water resources. However, the effluent must meet specific quality standards before being discharged to the river or stream, or being used for irrigation. While the return of effluents augments the quantity of water available downstream, it also affects the quality of the receiving water. Industrial, agricultural and urban effluents all contribute to the increase in salinity of the river, which is one of the major water quality issues facing South Africa (DWA, 1986).

1.2 WATER QUALITY MANAGEMENT IN SOUTH AFRICA

Water quality management embraces decisions and actions, which lead to the development, implementation and execution of strategies, to achieve its stated mission and objectives. The DWAF's objectives are to ensure that water of acceptable quality continues to be available for recognised users. Water quality is an important part of water resource management, and water quality management requires information based on monitoring procedures. DWAF's desirable goals are therefore to maintain water quality in a natural state, or in such a state that it remains "fit for use" for recognised users (DWAF, 1991). In terms of the National Water Act, the "fit for use" objectives are linked to a classification system, with resource objectives in classes A-D and E/F, where Classes E and F are degraded and degrading. The management tools that have been available to water quality managers include Uniform Effluent

Standards, (General, Special and Special Phosphate standards) for non-hazardous pollutants, the Pollution Prevention Approach for hazardous pollutants and the South African Water Quality Guidelines (SAWQG) (DWAF, 1996a-f). The General Effluent Standards have been widely used in water quality management in South Africa, and have the aim of assuring that any effluent discharged into a receiving water would meet both the minimum standards requirements and the SAWQG. However, in spite of nearly forty years of the implementation of effluent standards, the quality of many rivers has continued to deteriorate (Quibell *et al*., 1997).

At present, most water consumption without treatment poses a human health risk. Large-scale urbanisation of previously rural populations, plus growing industrialisation, has increased the water demand and the extent of impacts on water quality. The DWAF accepts that utilisation of water resources impacts on aquatic ecosystems, therefore it has to be regulated and managed so that the capacity of aquatic ecosystems to maintain their integrity is not irreparably reduced. The use of guidelines will therefore help to determine the degree to which the quality of receiving water could be altered, and protected.

In order to meet the growing needs of the country in terms of water demand and supply, sound management practices are essential. Setting water quality management criteria forms part of those management practices.

1.2.1 Uniform Effluent Standards approach

The Uniform Effluent Standards (UES) approach requires that effluents received by the natural environment comply with uniform standards, to control the input of various pollutants. The ultimate goal is therefore minimum pollution. In 1980, in an attempt to improve water quality, DWAF implemented the General, Special Effluent, and Special Phosphate Standards, which required all effluents released into the river to contain less than 1.0 mg/l of phosphates. The uniform standards are usually set to achieve pollutant concentrations in the effluent using the "best available technology not entailing excessive cost" (BATNEEC) to treat effluent (DWAF, 1991). Although the UES were thought to have been successful, water quality continued to deteriorate. This prompted the DWAF to advocate a change from the UES approach, which had mainly focused on effluent and ignored the impact of effluent discharges on the

quality of the receiving water, to the Receiving Water Quality Objectives (RWQO) approach (van der Merwe and Grobler, 1990).

The UES approach to water pollution control has two main advantages. It is simple, more understandable and is straightforward for regulators to enforce. The approach also has disadvantages. It may fail to protect the quality of water resources where there are multiple point sources of a particular pollutant or where there are high background levels arising from non-point sources. It is not cost-effective since it requires all effluent to comply with the same standards irrespective of the assimilative capacity of the receiving waters; it provides no incentive for industry to locate at the most advantageous environmental location; and it provides no framework for control of non-point sources (DWAF, 1991).

1.2.2 Receiving Water Quality Objective approach

The RWQO approach focuses on the quality of receiving water rather than the emission from the source (DWAF, 1996 a-f). The implementation of the RWQO approach aimed to manage the receiving water bodies in a state that is "fit for use" as defined by recognised water users, i.e. domestic supply, agricultural, recreational and industry (DWAF, 1996 a-d). In 1997, DWAF recognised the environment as the resource-base rather than as a "user" and resource protection, in order to sustain use, became the stated National Water Policy (DWAF, 1997a). Resource protection requires that point and diffuse sources be controlled to achieve the desired quality in the receiving water (DWAF, 1995). The RWQO approach recognised that the receiving water has the capacity to assimilate pollution without serious detriment to quality requirements of the recognised users, and has three main advantages (DWAF, 1991):

- Both point and non-point sources of pollution have to be taken into account as the focus is on the quality of the receiving waters and minimum interference with legitimate uses of the environment;
- it is cost effective as it optimizes the level of control required by considering the capacity of the receiving water environment to assimilate particular pollutants; and
- it offers an incentive for industry to locate where the receiving environment is

least sensitive to pollution.

The approach also has drawbacks, namely:

- Thorough understanding of the fate of pollutants and of their impacts on the water environment is needed;
- its application is technologically more demanding; and
- a more detailed investigation is required, since site-specific effluent standards have to be specified.

In cases where the RWQO approach is not appropriate, for example, handling and disposal of hazardous substances, the **Pollution Prevention** approach is preferred. The pollution prevention approach involves reduction and recycling to reduce the quantity and toxicity of waste and to minimise present and future threats posed by hazardous substances to the human health and the environment. Due to the toxicity, persistence and bioaccumulation of hazardous pollutants to the environment, the DWAF has adopted a **precautionary** approach (DWAF, 1991). The precautionary approach indicates that a positive action be taken to minimise undesirable impacts on the environment (DWAF, 1995). This approach strongly indicates that waste prevention is a valuable means of reducing the risks to the environment.

The RWQO approach has so far been applied mainly where polluters have asked for relaxation of the General or Special Standards. Although this approach can result in site-specific standards that are stricter than the General and Special Standards, all the cases that had been dealt with until 1991 have resulted in relaxation of the requirements (DWAF, 1991). In effect, water quality continues to be managed on the basis of UES. This has not really succeeded at all in protecting the quality of water resources, and it became necessary to implement another approach. This approach is the basis of sustainability in water resource use, embodied in the concept of the Reserve.

1.2.3 South African Water Quality Guidelines

As the custodian of South Africa's water resources, the DWAF's goal is to ensure the protection of the aquatic ecosystems so that they remain in a healthy and viable state, and that the quality of water resources remains "fit for use" (DWAF, 1996 a-f). This

is necessary as aquatic systems form the resource base from which other users are supplied (DWAF, 1996f; 1997a). The aim of developing South African Water Quality Guidelines (SAWQG) for Aquatic Ecosystems has been to develop a set of guidelines and criteria that are appropriate for ecological conditions in South Africa, based on a consensus amongst experts and water quality managers.

The guidelines contain values for a selected range of constituents expressed as Target Water Quality Range (TWQR), the Chronic Effect Value (CEV) and the Acute Effect Value (AEV), all of which are based of information of tolerances of aquatic biota. The TWQR is not a criterion, but a management tool (Roux *et al.,* 1996). DWAF's policy is to maintain constituents within the TWQR as part of its protective approach. The AEV is the concentration at and above which a statistically significant adverse effect is expected to occur after a short-term exposure, and the CEV is the concentration at and above which a statistically significant adverse effect is expected to occur after a long-term exposure (Roux *et al*., 1996). If chemical constituents at the CEV level persist in the aquatic ecosystems for indefinite periods, death of individuals and eventual disappearance of sensitive species from the ecosystems can be expected. Since there were very few toxicological data on freshwater species indigenous to South Africa, the AEV and CEV values in the SAWQG for Aquatic Ecosystems were derived from international toxicological databases. Species from different trophic levels and taxonomic groups were included (Moore, 1990; Moore *et al*., 1991; DWAF, 1996f). The lethal concentration or LC50 values (a concentration at which 50% of the test population die (Rand, 1995) of tolerance test results from a variety of taxa were used to calculate the AEV and CEV values. Safety factors were applied where data were limited. However, Parkhurst (1995) argued that the use of safety factors might lead to water quality standards and effluent limits that are more stringent and costlier than are necessary to protect aquatic life. In this study, safety factors were omitted and LC1 values were used to derive AEV values (Sections 3.5.5 and 4.5.5). Chronic values were not calculated, as chronic testing was not undertaken.

1.2.4 South African National Water Policy

The main principles of the National Water Policy are equity and sustainability (DWAF, 1997a). *Equity* means water must be available for everyone and *sustainability* means water utilisation forever. The aim of the policy is not to totally

prevent impacts, but to balance the long-term resource protection with short and medium term demands on water resources. Prevention of all impacts would hamper social and economic growth, but too little protection could lead to irreversible resource damage and limitations on future use. Therefore, the responsibility for water resource management is to protect water resources from over-utilisation or impacts, which cause degradation. The National Water Policy is given legal substance through the National Water Act (No. 36 of 1998).

The 1998 National Water Act protects any excessive burden on ecosystems, as they will deteriorate and lose the ability to sustain utilisation in the long-term. Freshwater ecosystems, the water resource base on which water users depend, need to be protected. It is the healthy functioning of the whole ecosystem which gives a water resource its ability to recover from unusual and stressful situations. For resource protection to be effective, **resource-directed measures** and **source-directed controls** have been developed (DWAF, 1997a).

Resource-directed measures

The existing effluent standards lack a link between the enforcing license conditions and the relevant state of the receiving environment (Palmer and Scherman, in press). Resource-directed objectives have four components, which are necessary for the protection of the resource-base namely:

- a classification system for water resources;
- determining an Ecological Management Class (EMC);
- determining the ecological Reserve; and
- setting water quality resource objectives for quality, quantity, biota and habitat (NWA, 1998).

The National River Health Programme (NRHP) is the first initiative to contribute to setting and monitoring resource objectives (Hohls, 1996; Uys *et al*., 1996).

The NRHP is based on a suite of instream bioassessment (biomonitoring) techniques, and one of the most widely used is the South African Scoring System Version 4.0 (SASS4) (Chutter, 1994; 1998). This method provides scores for the presence of selected invertebrate taxa. Low SASS4 scores indicate a water quality or habitat integrity problem, but do not indicate the cause of the problem. Toxicological studies

such as the ones undertaken in the present study provide complementary information, which can contribute to identifying the causes of low biomonitoring scores.

Source-directed controls

The RWQO approach was never comprehensively applied by the DWAF, and as a result, the quality of water resources has continued to deteriorate. Up to the present, most of the monitoring standards for licenses are based on chemical concentrations. Under the new National Water Act, stricter measures are being put in place to control the nature of wastewater discharged into water resources, thus protecting the environment. A key question is whether industries and sewage treatment works would be able to comply with the set standards considering a lack of modern technology and economic constraints? A more comprehensive approach to wastewater disposal, water conservation, human health protection and economic needs is an integrated approach. Because it is important not to be unnecessarily over-protective, the use of site-specific tolerance/toxicity testing in order to provide site-specific resource objectives can be economically advantageous.

Source-directed controls include all measures used to prevent or minimise wastewater discharges that might impact on the aquatic environment, for example: the issuing of end-of-pipe licenses; authorisation for water use; wastewater standards for point-source pollutants, and appropriate technological changes (DWAF, 1997a). With the rapid developments in toxicity testing proving successful, toxicity end-points could be included in future license requirements, contributing generally to source-directed measures.

1.3 ECOTOXICOLOGY

Ecotoxicology is an interdisciplinary science, integrating toxicology with environmental chemistry and ecology (Rand and Petrocelli, 1985; van der Gaag, 1991). It was introduced because of growing concern about the effects of environmental chemicals upon ecosystems. Ecotoxicology investigates the effects of toxic substances already present in the environment and aims to predict the effects of newly introduced chemicals (van der Gaag, 1991). It deals with harmful effects of chemicals within the context of ecology (Walker *et al*., 1996), and includes the

interaction of chemical substances with the physical environment in which the organisms live (Rand, 1995). Where the focus of a study is more on producing laboratory data, as in this study, and integrating ecological studies are not undertaken, the study remains in the discipline of aquatic toxicology. In this study, a level of environmental "realism" is provided by the use of wild populations of riverine invertebrates as test taxa.

1.3.1 Aquatic toxicology

Aquatic toxicology is a multi-disciplinary science that studies the chemical, physical and biological factors that affect environmental concentrations of chemicals (Sloof, 1988; Rand *et al*., 1995), and can contribute substantially to the protection of natural ecosystems (Cairns and Mount, 1990). Its main function is to identify chemicals that can have adverse effects on aquatic organisms (Rand, 1995), and it can be applied in Ecological Risk Assessment (ERA), Whole Effluent Toxicity (WET) evaluations and in the derivation of water quality criteria. In this study, aquatic toxicology is used in site-specific whole effluent toxicity evaluations.

Aquatic toxicology can be undertaken using single or multi-species systems. Most single-species tests are conducted in the laboratory and provide information on the duration of exposure that produces changes in factors such as mortality, growth and reproduction within the species. Single-species tests use one species from any trophic level. They are simple, easy to conduct and can be replicated, but do not account for the adaptive ability of natural populations of organisms. The single-species approach has been criticised as it ignores the fact that a pollutant does not only affect single organisms, but also higher levels of the biological hierarchy (Cairns, 1986; 1992). Multiple-species tests can be conducted in the laboratory and provide information more predictive of ecological consequences of the chemical release. Despite the criticisms, single-species tests seem to remain the major regulatory tool upon which decision-making and management is based (Rand, 1995). In this study, single-species testing was undertaken.

Aquatic toxicity tests have been conducted using aquatic species like fish and *Daphnia* spp. in single-species tests. Fish have traditionally been the most common organism used in aquatic toxicology, because:

- they were viewed as an important resource requiring protection;
- it was thought that their high sensitivity to pollutants would ensure that standards derived for their protection would automatically serve for the protection of less sensitive aquatic biota; and
- several species were available in a disease-free state from artificial culture (Boudou and Ribeyre, 1989).

However, macro-invertebrates and diatoms have been found to be more sensitive to lower concentrations of toxicants than fish (APHA, 1992), and often represent a large majority of the biomass in a natural system (Buikema *et al*., 1982).

Daphnia spp., a small invertebrate commonly called a "water flea", has been widely used as a standard test organism for toxicity testing (Rand and Petrocelli, 1985). However, *Daphnia* spp. can only be found in standing waters such as dams and lakes. Most point-source pollution occurs in rivers, i.e. flowing water systems, and in South Africa rivers are the most important freshwater aquatic ecosystems (DWAF, 1997a). There is therefore a need to produce data on how indigenous riverine invertebrates respond to potential pollutants, both as single substances and as complex effluents.

The application of aquatic toxicology in South African water resource management is relatively new. Toxicity tests have not yet been required for regulatory purposes, although guidelines for drinking water have been recently developed using toxicity testing methods (Slabbert *et al*., 1998 a, b). The tests were conducted using *Daphnia*, protozoa, algae and enzymes, but not indigenous invertebrates, which are the resident organisms in the aquatic environment. Indigenous riverine invertebrates are relevant to the development of resource objectives for ecosystems.

In this study, indigenous riverine aquatic macroinvertebrates were used as test organisms. There is a need for such toxicity data, as it is not known how the standard test species such as *Daphnia* and international toxicity data compare to the responses of local species. Therefore, it is difficult to evaluate the real level of protection afforded by the SAWQG (DWAF, 1996f). Indigenous organisms increase both the reliability of data and the environment realism. Therefore, the use of indigenous riverine organism could be particularly useful in setting site-specific guidelines. There

is also a need for the development of standardised test protocol for indigenous organisms. The Centre for Aquatic Toxicology (CAT) at the Institute for Water Research (IWR) has developed a protocol for acute toxicity testing using selected indigenous riverine invertebrates in artificial stream systems (DWAF 2000).

1.3.2 Toxicity testing

Biological toxicity testing has become an increasingly important approach in assessing potential effects on aquatic biota, as chemical/physical tests alone are not sufficient to ensure resource protection (APHA, 1992). Toxicity testing can be used to determine tolerances of riverine organisms to single-substances, such as salts and specific ions, as well as whole-effluents that may be saline. It can cope with variations in the composition of complex effluents and detect the effects of the combination of all compounds present (Hunt *et al*., 1992). Toxicity testing has been incorporated into the issuing of licenses, resulting in the improved control of toxic discharges (Owens, 1991). Its application in the surveillance and monitoring of inputs to the aquatic environment is widespread and common throughout the USA and Europe and to a limited extent in Australia (Wall and Hanmer, 1987; Richardson and Martin, 1994). A toxicity-based approach is well used in the USA to identify environmental problems, establish regulatory priorities, set permit limits, and to monitor unacceptable effluent effects. In South Africa, toxicity testing has not yet been required for regulatory purposes. Since toxicity results provide data on biotic responses to chemical concentrations, toxicity data provide a link between biological and chemical monitoring data.

The information gained from various toxicity tests can be of use in pollution management for:

- *prediction* of the environmental effects of a waste product;
- comparison of toxicant effects on animals; and
- regulation of discharges (Buikema *et al*., 1982).

Toxicity test data can also provide information about the mechanisms of toxicity, synergistic or antagonistic interactions with various environmental parameters, and the overall impact of stresses (Coler and Rockwood, 1989). Toxicity testing can be conducted in a controlled laboratory with a limited number of variables, in a natural

ecosystem, and in experimental model ecosystems (simulated indoor or outdoor) (Rand, 1995). In this study, toxicity tests were conducted in the laboratory.

Toxicity tests procedures are typically classified according to:

- duration (i.e. short-term or long-term);
- method of adding the test solution (i.e. flow conditions); and
- the purpose of the test (i.e. toxicity endpoint) (Rand, 1995).

The endpoints are often related to the duration of exposure to the test solution and the life stages of the exposed organisms (Buikema *et al.*, 1982; Rand, 1995). Tests can be lethal (where the end-point is death) or sub-lethal (where the end-points could be changes in growth, reproduction or behaviour). In lethal testing, the lethal concentration or LC50, a concentration at which 50% of the test population die, is the standard response (Rand, 1995). Acute tests (short-term tests i.e. 96 hrs or less) may be undertaken in systems that are static, renewed, recirculated, or flow-through. Any technique to be used is dependent on the specific test and the objective of the test. In static tests, test organisms are placed in test chambers with the toxicant solution and kept there for the duration of the test. Static tests have the following characteristics:

- test organisms are exposed to the same toxicant mixture for the whole test;
- lentic organisms are suitable test taxa;
- tests are simple and inexpensive, using small volumes of water;
- toxicant concentration may decrease during the test; and
- oxygen levels may drop, while excretory toxin levels may rise (Pascoe and Edwards, 1989; US EPA, 1992; Rand, 1995).

Some of the disadvantages of static tests are overcome in static renewal tests in which test solutions are replaced, usually at 24 hour intervals (Rand, 1995).

Recirculation tests have test water that is flowing, but is recirculated through the test chamber during the test. A flow-through test is when there is a continuous, once only, flow through the test chamber during the test. A flow-through test system prevents metabolite build-up and interaction with toxic substances; reduces the loss of toxic substances to adsorption and volatilisation (Pascoe and Edwards, 1989); and the dissolved oxygen (DO) levels remain high (US EPA, 1992). However, this system does not provide information about the persistence of toxicity (US EPA, 1992), and

uses large quantities of water. Flow-through tests are found to be cumbersome, expensive to operate, and create large quantities of wastewater (Williams *et al*., 1984; US EPA, 1992). Although it has been suggested that if the test is to determine the toxicity of wastewater to riverine organisms, then a continuous flow test would be most appropriate (Buikema *et al*., 1982), recirculation test systems were used for this study to avoid problems of water supply and waste disposal.

Acute toxicity tests

Acute toxicity tests have been used extensively to determine the effects of potentially toxic materials on aquatic organisms during short-term (usually 96 hours or less) exposure under controlled conditions (Parrish, 1985; Van Leeuwen, 1988a). Acute toxicity tests are conducted by exposing groups of organisms to different concentrations of test material or effluent concentrations. The response produced is usually death and its criteria are lack of movement and of reaction to prodding (Sprague, 1973; Parrish, 1985). Acute toxicity tests are simple to conduct, and are easily interpreted. In this study, acute toxicity tests were used to investigate the effects of saline effluents to riverine macroinvertebrates, using recirculating artificial stream systems.

Chronic toxicity tests

Acute toxicity testing may not indicate toxicity but that does not necessarily mean test water is not toxic. Chronic toxicity tests evaluate the possible toxic effects of a test material under long-term conditions, at lethal and sub-lethal concentrations. In chronic tests, the test organism is generally exposed for an entire reproductive lifecycle or partial-cycle to at least five concentrations of the test material (Rand and Petrocelli, 1985; Van Leeuwen, 1988b). The duration of chronic toxicity testing ranges from 7 (sub-chronic) or 10 days (short-term chronic), to months (chronic). Chronic test end-points involve long-term mortality, changes in growth, reproduction, survival and behaviour (Rand and Petrocelli, 1985; Van Leeuwen, 1988b; Rand, 1995). Therefore, chronic tests can provide a more sensitive measure of chemical toxicity than acute toxicity tests (Rand, 1995). In this study, limited chronic toxicity testing was undertaken.

1.3.3 Whole Effluent Toxicity (WET) Testing

Many industries discharge their effluents as complex chemical mixtures with their synergistic, antagonistic and additive characteristics, into water resources. The complexity of effluents makes it difficult and expensive to identify individual chemicals. In South Africa, discharge of untreated industrial effluents into the environment either directly or via municipal sewage treatment works, is causing water quality problems to the limited water resources. It is therefore important that wastewater be treated to satisfactory standards before being discharged into the receiving water.

WET testing was formulated with the intention to identify, characterise and eliminate toxic effects of discharges on water resources. It has been a useful tool for identifying toxicity impacts on the environment (Grothe *et al*., 1996). WET testing forms a major portion of the US EPA's integrated approach to toxins. Its major objective is to estimate the "no adverse effect" concentration of effluents or pure compounds, thus protecting the environment (Chapman *et al*., 1996). It is most appropriate in situations where:

- effluent constituents are not completely known;
- a complex mixture of potentially synergistic, additive and antagonistic toxic pollutants are discharged into the environment;
- more than one discharger is located in a specific area and the potential exists for effluent mixing and additive toxic effects; and
- a chemical-specific evaluation is not practical due to lack of information about toxic effects of a chemical or lack of resources required to model the chemical(s) present (US EPA, 1985a).

The DWAF has identified WET testing as a tool to evaluate the suitability of hazardous effluents for discharge into receiving waters (Slabbert *et al*., 1998a). Chemical analysis can be costly when complex organic substances are involved, and WET testing can be a cost-effective route to determining the dilution of the whole effluent required and associated toxic effects (Palmer and Scherman, in press). However, when this approach is adapted to using indigenous, wild test populations,

there is the added challenge of variability of the wild population, as well as the variability of effluent composition.

Effluent variability

Several factors should be considered in making the choice of toxicity test system, e.g. is the effluent highly variable and is the discharge continuous or intermittent? If the effluent is variable and continuous, then a continuous, flow-through test is preferred. If it is variable and intermittent, static renewal is preferred and a composite test sample is used (Burton *et al*., 1996). Effluent variability is caused mainly by changes in the composition of the effluent. If the effluent is not variable, e.g. the effluent is discharged from a retention dam, then a static or renewal system and a grab sample are appropriate for the test (Rand, 1995).

Effluent sample variability

The effluent sample should be representative of the whole effluent to be tested. Effluent can be kept frozen if not used immediately after collection, but must be defrosted and the temperature adjusted before use. Effluent may be coarse-filtered through a sieve to remove any large floating particles or suspended solids before it is used, however, this must be done with caution as filtering may reduce toxicity (US EPA, 1993).

A grab sample may be completely adequate in the case of an effluent that varies little in composition through time, but may be completely inappropriate for characterising the toxicity of an effluent that varies over time. A grab sample is recommended for acute toxicity testing, but will not reveal extreme situations. For example, a sample will only show a toxicity peak if the sample has been collected during a toxicity peak period. One of the advantages of using a grab sample is that the effluent toxicant concentrations remain relatively constant throughout the test duration (Burton Jr. *et al*., 1996). A composite sample may prove ideal for chronic toxicity tests, but significant variability will exist within the effluent sample (Eagleson *et al.,* 1986). This method may mask periods of peak toxicity (Keith, 1990; US EPA, 1992), as a composite sample tends to average chemical constituent fluctuations during the sampling period (Eagleson *et al.,* 1986). Composite samples are recommended for chronic toxicity tests where peak toxicity of short duration is not critical.

For this study, 2 types of whole effluent were tested, and their characteristics are discussed in Chapters 3 and 4 respectively:

1) pulp and paper kraft 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 collected into a holding dam after irrigation).

In conclusion, WET testing potentially measures (US EPA, 1992):

- the effects of those toxic substances that are present in a form that can affect organisms, and
- the effects of interactions of constituents.

However, it also has the following potential disadvantages (US EPA, 1992):

- it provides no information about protecting human health;
- it does not indicate how long toxicity persists in the environment; and
- it does not take into account the changes in toxicity that can result from environmental changes.

End-points

For acute toxicity tests, the most commonly used end-points are mortality and immobilisation (Rand and Petrocelli, 1985; APHA, 1992). The median effective concentration (EC50) may therefore be cited as the end-point rather than median lethal concentration (LC50), when mortality is difficult to define (APHA, 1992). The EC50 is the estimated concentration of the toxicant which will have an effect on 50% of the test population. In this study, mortality was the selected end-point and LC50s were determined.

1.3.4 Artificial stream systems

Artificial stream systems have been used in ecotoxicological research to recreate and mimic natural conditions in rivers, under controlled environmental conditions (Shriner and Gregory, 1984; Kosinski, 1989; Lamberti and Steinman, 1993). They have been

used to study the basic ecological principles of lotic ecosystems. They have also been used to investigate the effects of toxic chemicals on specific components of the ecosystem and the interactions among and between species and their environment (Kosinski, 1989; Lamberti and Steinman, 1993). There are outdoor and indoor artificial stream systems of different scales.

The CAT-IWR in Grahamstown, has undertaken artificial stream research since 1992. The aim of their research is to employ the experimental methods of aquatic toxicology to explore the possibilities of conducting experiments using indigenous riverine organisms (Palmer *et al*., 1996; Williams, 1996; Binder, 1999; Everitt, 1999; DWAF, 2000). In this study, investigations into the tolerances of selected indigenous riverine macroinvertebrates to complex saline kraft and textile effluents were undertaken.

1.3.5 Test organisms

The use of indigenous species in toxicity testing improves the ability to predict responses in the field, however, it may be problematic to collect and maintain test species in the laboratory, especially early instars of highly sensitive groups e.g. mayflies (Clements and Kiffeney, 1996). The size and life stage may cause under or over-estimation of effects in the field. Organism identification is important as results can be expressed at the level of the individual organism's response, or community level response. This expression can be in terms of biomass, species richness, diversity or functional group biomass (Guckert, 1993).

Generally, younger organisms are considered more sensitive to toxic stressors than older organisms. The contributing factors are frequent moulting during early life stages in many invertebrates, surface/mass ratio and organ tissue formation (US EPA, 1993; Burton Jr. *et al*., 1996). Mayfly nymphs were chosen as test organisms for this study, as they are sensitive (Williams, 1996; Clements and Kiffeney, 1996) and play an important role in river ecosystem function (Davies *et al*., 1993; Davies and Day, 1986; Palmer *et al*., 1993). The test organisms used were *Tricorythus tinctus*, a filter-feeding tricorythid mayfly from the reference site in the Sabie River (Palmer and Scherman, in press), and a mixed baetid population from an unimpacted reach of the Buffalo River. Test organisms were collected from the same area, as organisms of the same species from different sources may have different sensitivities to the same

toxicant (Weber, 1981; APHA, 1992; Chapman, 2000), which would increase the variability of test results (Rand, 1995). Both species are fairly widespread in many rivers in South Africa and are both abundant components of the invertebrate fauna (Palmer *et al*., 1993).

1.3.6 Aquatic toxicology at the Centre for Aquatic Toxicology – Institute for Water Research, Rhodes University

Artificial stream laboratory has been developed at CAT-IWR for investigations into the tolerances of South African riverine invertebrates. The artificial streams provide lotic conditions, and allow comparisons with the responses of the lentic *D.pulex*. Selected riverine macro-invertebrates have been exposed to water quality variables, such as various salts, metals and chlorine with the aim of contributing to the development of water quality guidelines for the natural environment (Palmer *et al*., 1996; Goetsch and Palmer, 1997; Gerhardt and Palmer, 1998; Binder, 1999; Everitt, 1999). Test taxa are usually collected from rivers, but investigations into laboratory rearing have been undertaken (Haigh and Davies-Coleman, 1997). The CAT-IWR is also involved with whole effluent toxicity (WET) testing using complex effluents. This study forms part of the WET testing programme (Palmer and Scherman, in press).

1.4 LIMITATIONS OF THE WET APPROACH

This research acknowledges several general limitations inherent in WET testing (Grothe *et al*., 1996; US EPA, 1992):

- Test results are variable and not always reproducible.
- If effluent toxicity degrades rapidly in the receiving water, WET test results may over-estimate effects to resident biota (Parkhurst and Mount, 1991).
- Properties related to specific chemicals in complex effluents (such as bioaccumulation and carcinogenicity) are not generally assessed.
- Where there are chemical/physical conditions present that act on toxicants in such a way as to "release" toxicity downstream, such toxicity may not be measured in the effluent (US EPA, 1985b).
- WET testing only provides an indication of the toxicity of a solution and does not

identify the specific toxic components, nor is it indicative of the complexity of the mixture (Hunt *et. al.*, 1992).

1.5 ECOLOGICAL RISK ASSESSMENT

Ecological Risk Assessment (ERA) is a new tool in South Africa, and serves to support sustainable environmental management. ERA describes the probability of a hazardous substance affecting an ecosystem, as risk is defined as the probability that a hazard will be realised (Chapman, 2000). ERA has two basic elements:

- exposure, which is the interaction of stressors and receptors, and
- an analysis of effects, which evaluates changes in the nature and magnitude of effects

  as exposure changes.

Integrating exposure and effects information leads to an estimation of risk (SETAC, 1997). The approach provides an objective way of balancing the degree of risk to be permitted, against the cost of risk reduction and competing risks (Palmer and Scherman, in press). In this study, the level of environmental hazard posed by different dilutions of effluent is ranked, and related to the classification (DWAF, 1997a) of the river. As mentioned in Section 1.3.3, WET is a tool to identify a hazard, and hazard identification is the first stage of ERA.

1.6 SALINISATION

Salinisation is seen as one of the single most serious threats facing public water use in South Africa (DWA, 1986; DWAF, 1997b), as it renders water unsuitable for many uses. Saline effluents from industries and sewage treatment works also contribute to the high salinity of receiving waters. Increased water recycling within the catchment can also cause conservative components such as chloride to build up in surface and groundwater as a result of evaporation. Chloride and sulphate anions and sodium cations are most commonly implicated in salinisation (DWA, 1986). In this study, the focus was on salinisation due to the discharge of saline effluents into the environment.

Spray irrigation can lead to a build-up of naturally-occurring salts in the soil as the water evaporates, forming more concentrated salt solutions which percolate through the soil leaching more soil salts (Williams, 1987). This results in increasing salt loads in the nearby stream, river or groundwater. The salt then percolates down into the groundwater or runs downstream and finally into the rivers. Continuous irrigation, with evapo-transpiration of saline water, results in the accumulation of salt in the upper soil layers, which can seriously affect plant growth and crop yields (DWAF, 1996c). This may ultimately result in irreversible damage to the soil structure since high sodium concentrations damage the clay particle structure and soil permeability. Discharges of saline industrial and sewage effluents are a common cause of increasing salinisation and deteriorating water quality. Some industries have therefore considered desalinisation processes, so that treated effluents could be re-cycled, reducing the mineral enrichment of the receiving waters. However, the high salinity of some industrial effluents has limited the industrial recycling of wastewater.

1.7 AIMS AND OBJECTIVES OF THE STUDY

The aim of this study was 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 six objectives.

Objectives

- To establish a capacity for toxicity testing using flowing water organisms.
- To identify suitable test organism(s) and to conduct tolerance experiments using these test taxa.
- To contribute to the environmental water quality objectives for kraft (pulp and paper) 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 CAT-IWR.

1.8 THESIS STRUCTURE

CHAPTER 1 **TOXICOLOGY AND WATER QUALITY MANAGEMENT IN SOUTH AFRICA**

This Chapter describes the water quality management of South Africa's water resources with emphasis on the new National Water Act and the South African Water Quality Guidelines. It introduces the use of instream bioassessment and toxicity testing as tools for water quality management. Emphasis is placed on WET testing as a tool that is currently undergoing method development. Ecological Risk Assessment is introduced, and salinisation is mentioned briefly as industries are contributing to salinisation via the discharge of saline effluents into receiving waters. Aims and objectives of the study are listed.

CHAPTER 2 **MATERIALS AND METHODS**

This Chapter describes the general methods used in toxicity testing applicable to both kraft and textile effluents. Artificial recirculating systems are described. Collecting and handling of effluents, selecting and collecting of organisms, selection of dilution media, laboratory design, experimental design and procedure are explained. Data processing is discussed. Methods specific to each effluent are covered in Chapters3 and 4 respectively.

CHAPTER 3 **EFFECTS OF KRAFT MILL EFFLUENTS ON NYMPHS OF THE MAYFLY *Tricorythus tinctus* Kimmins FROM THE SABIE RIVER, MPUMALANGA**

A general description of kraft mill pulp and paper effluent is given. Details of the manufacturing process with special reference to a Mpumalanga mill are explained. A general review of the effects of kraft effluents on freshwater aquatic environments is given, which serves to highlight the northern hemisphere focus on the responses of fish, macro-invertebrates and algae. The specific aims and objectives of the study are introduced and a brief description of the study site and specific methodology used during the study is given.

A single test population of the mayfly nymph *T. tintus* was exposed to general and irrigation effluent. Whole effluent toxicity results and their application in the water quality management of both the Elands River (receiving water) and the Sabie River (source of test organisms) are discussed.

CHAPTER 4 INVESTIGATING THE EFFECTS OF TEXTILE EFFLUENTS ON MAYFLY NYMPHS FROM THE BUFFALO RIVER, EASTERN CAPE

This specific study investigated the effects of textile mill effluent on riffle-dwelling macro-invertebrates from the Buffalo River, using effluent from an Eastern Cape textile mill. The South African textile industry, the selected Eastern Cape textile mill, and the study area are briefly discussed. Methods specific to the effluent and test organisms are described, and mortality data presented. Mayfly nymphs were exposed to a wide range of effluent concentrations (general and post-irrigation effluents) over 96 hours and 7 days. The application of the toxicity data in water quality management of the Buffalo River is discussed.

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

In addition to concluding remarks and recommendations are made in the context of WET testing method development, and its application in water resource management.

CHAPTER 2

MATERIALS AND METHODS

Chapter 2 describes general methods, applicable to both kraft and textile effluent experiments. Additional specific information can be found in Chapters 3 and 4 respectively.

2.1 INTRODUCTION

Wastewater from industries and municipalities is a major source of pollution to aquatic environments. The management of toxic substances entering natural waters is complex, as pollutants often enter in a diffuse manner as multiple sources. This disposal of increasing amounts of toxic chemicals has created a need for information on the fate, transport and effects of these substances in the environment. At the same time, demands of urbanisation and economic activity have compromised the assimilative capacity of scarce water resources. Strict measures must therefore be applied to reduce pollution of the aquatic environment. Assimilative capacity is the ability of the receiving water to dilute or degrade the pollutant without damage to the aquatic environment (Grothe *et al*., 1996). The first step in trying to maintain assimilative capacity is to determine what effluent concentrations can be allowed to enter the river, that is, to determine the toxicity of the effluent. Whole effluent toxicity (WET) testing is an ideal tool for this process (Slabbert *et al*., 1998a).

The control of point source discharges from industries and municipal wastewater treatment facilities has resulted in continuous improvements to the quality of receiving waters of the United States of America (USA) (Dorn and van Compernolle, 1995). In South Africa, the lack of enforcement of point source regulation has resulted in many water resources being impacted. Industrial effluents are complex and variable and may contain substances that, in combination, may have synergistic or antagonistic effects (Weber, 1981). The composition of effluent and relative concentrations of the components also changes as it moves through the receiving water (US EPA, 1986), so

that the toxicity of the effluent downstream may not be the same as at the discharge point, although it is the same effluent.

Many countries are currently applying biological toxicity testing methods to monitor and control the discharge of harmful substances into the aquatic environment. In the USA, the Environmental Protection Agency (EPA) uses an integrated hazard assessment approach in which biological toxicity testing plays an important role (US EPA, 1989). The Organisation for Economic Co-operation and Development (OECD) also advocates the use of toxicity testing to control toxicants in industrial effluents and related receiving waters (OECD, 1987). In South Africa, there are a number of institutions, e.g. Environmentek of the Centre for Scientific and Industrial Research (CSIR), the Department of Water Affairs and Forestry's (DWAF) Institute for Water Quality Studies (IWQS), Rand Water, Sasol and the CAT-IWR, that have the infrastructure and facilities to conduct freshwater toxicity tests.

Toxicity testing has been in use for water and wastewater testing in South Africa since the early 1980's (Grabow *et al*., 1985; Slabbert, 1988). The bioassay procedures for routine testing in South Africa use standard organisms e.g. *Daphnia pulex*. These procedures have been developed by the CSIR and IWQS and are similar to those applied by other countries (Slabbert *et al*., 1998 a,b). However, the DWAF's decision to incorporate WET testing into its toxic effluent management policy has necessitated the establishment of appropriate procedures for use in a South African context (Slabbert *et al*., 1998a). The focus has been on the use of standard laboratory organisms. However, this study focuses on the use of WET testing to contribute to the development of methodologies using riverine indigenous macroinvertebrates as test organisms.

In this Chapter, the recirculating artificial streams used for this study are introduced, WET testing methodology, how selected test effluents were collected and handled, and the selection and collection of test organisms are discussed briefly. The experimental design and procedure, and statistical analyses are also explained.

2.2 EFFLUENT COLLECTION AND DILUTION SERIES

In this study kraft (Chapter 3) and textile mill (Chapter 4) effluents were chosen as test effluents, as they are saline and contribute to the salinity problem in receiving waters. In South Africa, salinisation is seen as a serious threat facing public water use, and is a pressing water quality problem (DWA, 1986; DWAF, 1997b). Neither the kraft or textile mill effluents used in this study are discharged directly into a river, instead the effluents are used for irrigating kikuyu pastures. The effluents do however enter the river via overflow run-off and/or groundwater seepage. Neither effluent undergoes secondary treatment before irrigation.

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). Preliminary chemical analyses of the textile effluent by IWQS suggested little variation in composition (Tables 4.2). Grab effluent samples were also more practical to collect, particularly for the kraft effluent. Effluent test samples were collected and transported in clean plastic containers to avoid sample contamination. Containers were filled and closed tightly to minimise aeration during transportation, which could 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*., 1998a). The effluent concentration series used in this study was usually 1, 3, 10, 30 and 100%. This selection of concentrations follows that used by the US EPA (1985a; 1989; 1993), which recommends a geometric dilution series; that is, exposing animals to progressively increasing concentrations of a toxicant at a particular dilution factor, to elicit a response. Fifty and 75% effluent concentrations were added in some cases to increase the range of concentrations. The range of concentrations was also modified based on the results of the previous experiments. Effluent was coarse-filtered through a sieve (US EPA, 1993) to remove any large floating particles or suspended solids that may clog the filter.

2.3 TEST ORGANISM COLLECTION

All test organisms were collected in fast flowing riffle habitats, from the same area of the selected rivers. Organisms of the same species but from different sources may have different sensitivities to the same toxicant (Weber, 1981), thus increasing the variability of test results (Rand, 1995). Collection was carried out from the end of the riffle moving upwards through the sampling site, to prevent disturbing the site. 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 Baetidae, by rinsing rocks into a jug of river water, as they easily let loose of their hold on rocks. Test organisms could not be identified to species levels during collection, and were identified at the termination of the toxicity experiments. The Tricorythidae comprised a single species, *Tricorythus tinctus,* but the Baetidae were a species complex, dominated by *Baetis harrisoni* and *Afroptilum parvum*. This was a disadvantage as different species from one family may exhibit different sensitivities to the same toxicant (Hoekstra, 1991; Rand *et al*., 1995; US EPA, 2000), often resulting in a non-monotonic concentration-response relationship for the family.

Only organisms that appeared healthy were used for the experiments. Care was taken not to injure organisms during collection and transportation, so as to minimize variation in responses related to handling. Organisms were kept in a cooler box with aerated river water during collection and transportation. Ice packs were used to keep the water temperature from rising, when necessary. Pieces of sponge and leaves were placed into the cooler box for organisms to cling onto. Once enough organisms were collected, they were taken directly to the laboratory for sorting. Details regarding sorting organisms can be found in Chapters 3 and 4. Baetids were identified to species level at the end of each experiment; a percentage frequency table is presented in Chapter 4 for the Baetidae.

Organisms of similar size (those within a size range of 50% of one another (Coler and Rockwood, 1989)), age group and life stage were used for testing (APHA, 1992). Animals were carefully observed for signs of distress, physical damage or immobility. If any of these signs were identified, the organism was not used as it was pre-stressed (Coler and Rockwood, 1989). The test animals were not fed during the experiments to

reduce the variability due to nutritional and metabolic status (Parrish, 1985). APHA (1992) states that animals should not be fed during 96 hour short-term tests, and this is usually the no-feeding limit for macro-invertebrates (Buikema and Voshell, 1993).

2.4 LABORATORY DESIGN

Toxicity tests were conducted under semi-controlled laboratory conditions at the Rivers Research laboratory in Skukuza (Kruger National Park, Mpumalanga) for the kraft effluent, and the Zwelitsha Scientific Services laboratory in the Eastern Cape for the textile effluent. Temperature control is critical during toxicity experiments, as aquatic insect larvae are sensitive to high temperatures and the rate of emergence increases. Higher temperatures therefore increase the metabolic rate of the organism, facilitating more rapid toxicant degradation (Buikema and Benfield, 1978). Laboratory temperatures were controlled with the use of air-conditioners to around 20°C (see Chapters 3 and 4 for more detail). Light is also a significant variable affecting an organism's sensitivity to toxicants (Buikema and Voshell, 1993). Lighting was maintained at a 12:12 hour light:dark cycle using OSRAM biolux tubes providing a spectrum of wavelengths similar to sunlight (DWAF, 2000).

2.5 EXPERIMENTAL DESIGN

There are two common experimental designs used in toxicity testing, i.e. Analysis of Variance (ANOVA) and regression approaches. An ANOVA approach uses replication, and accounts for variability in a population's response to a toxicant (Stephan and Rogers, 1985; APHA, 1992; Ellersieck and La Point, 1995). If the response around the concentration-response curve is not monotonic, the ANOVA can incorporate this variability as the method compares the means of the different replicates. However, each concentration must have at least two or more replicates (APHA, 1992), and must be independent of each other.

Acute toxicity tests are generally based on an unreplicated regression design, where groups of test organisms are exposed to progressively increasing concentrations of a toxicant, in this case effluent (Gelber *et al*., 1985). Regression design is used to

estimate cause-and-effect type relationships, meaning that it estimates the relationship between the toxicant and test organism. In this study, most experiments were conducted using a regression design with one channel at each effluent concentration, and a control. A wide range of dilutions was mostly used as they had greater value than few replicated dilutions. The strength of the statistical estimate of the median lethal concentration (LC50), increases with an increased number of dilutions (Guckert, 1993). This produces a toxicity curve that describes the concentration-response relationship between the toxicant and the test organism.

Irrigated effluents influence river water quality via the groundwater. At the Mpumalanga mill site, groundwater surfaces as a spring between the irrigated fields and the river. Test organisms were exposed to duplicate mixtures of river water and groundwater (Chapter 3).

The overall experimental design consisted of testing Ephemeropteran mayfly nymphs for 96 hours to a number of progressively increasing effluent concentrations plus a control, in recirculating channels. All toxicity tests included a control to ensure that the effects observed are associated with, or attributed to exposure to the test media. Controls also provide a baseline and a point of correction for interpreting the results (Rand *et al.*, 1995). They are therefore used to determine the effects related to the health of the organisms and the quality of the dilution water.

2.6 DILUTION MEDIUM

River water was preferred as the dilution medium, as the purpose of the tests was to determine the sensitivity of indigenous site-specific organisms to selected effluents (Buikema *et al*., 1982; Guckert, 1993). According to Cooney (1995), if the objective of the study is to estimate the toxicity of test substance such as effluents, then the water sample should be representative of the receiving water. The interactions between the water and the toxicant are therefore incorporated into the end-point, and the relative toxicity of the toxicant is determined.

River water also contains detritus from which the organisms can feed during the experiment, and provides organisms with the necessary concentrations of nutrients,

minerals and suspended solids (Guckert, 1993). However, clearing the output end of the channels with a fine brush, as well as washing pump intake sponges, reduced the accumulation of debris and faeces in the channel systems used during this study and aided water flow around the streams. The use of very turbid water was avoided as it forms deposits and clogs the system. As the quality of dilution water can affect the toxicity of test effluents by altering the bioavailability of toxicants to the organism (Grothe *et al*., 1996), test water was collected from the Buffalo and Sabie Rivers from unimpacted flowing water areas.

2.7 EXPERIMENTAL SYSTEMS : RECIRCULATING ARTIFICIAL STREAMS

Artificial stream systems have been developed for toxicity testing to provide controlled flowing water environments, where riverine organisms from local rivers can be subjected experimentally to changes in water quality (McCahon and Pascoe, 1990; Guckett, 1993; Palmer *et al*., 1996). Artificial streams have therefore been widely used to simulate natural conditions, and as models of stream ecosystems to investigate a wide range of organisms, populations, community and ecosystem characteristics and functions (Shriner and Gregory, 1984; Kosinski, 1989; Lamberti and Steinman, 1993). One of the advantages of using artificial streams is that they can be replicated, are easier to manipulate than natural systems (Kosinski, 1989). They represent unique properties of flowing water systems not present and evaluated in lentic systems (Graney *et al*., 1995). An additional advantage of using artificial streams rather than an *in situ* stream is the prevention of harm to the environment via wastewater production.

In this study, experimental flowing water systems were used, as test organisms are riffle-dwelling mayflies that are in constant contact with flowing water. The streams used were recirculating systems, requiring smaller quantities of river water (i.e. dilution medium) to be transported to the laboratory, and generating smaller volumes of wastewater. Recirculation also ensured good oxygenation of the water. Williams (1996) first designed a flow-through version of the experimental stream systems used in this study, during her research on the responses of macroinvertebrate communities and species to chlorinated sewage effluents.

Each artificial stream, referred to in this study as a *channel* (Figure 2.1), was made of 1m long, 0.14m wide and 0.08m deep white polyvinylchloride (PVC) round-bottomed guttering, which is non-absorbent and highly resistant to chemicals. This ensured that the water quality was unaffected by the materials (Shriner and Gregory, 1984). A short channel length was preferred as it promotes rapid and homogenous mixing of the whole effluent and dilution medium down the channel. If toxicant pulses pass through a long large stream, the toxicant concentration may be reduced by the time it reaches the other end (Brooks *et al.*, 1996).

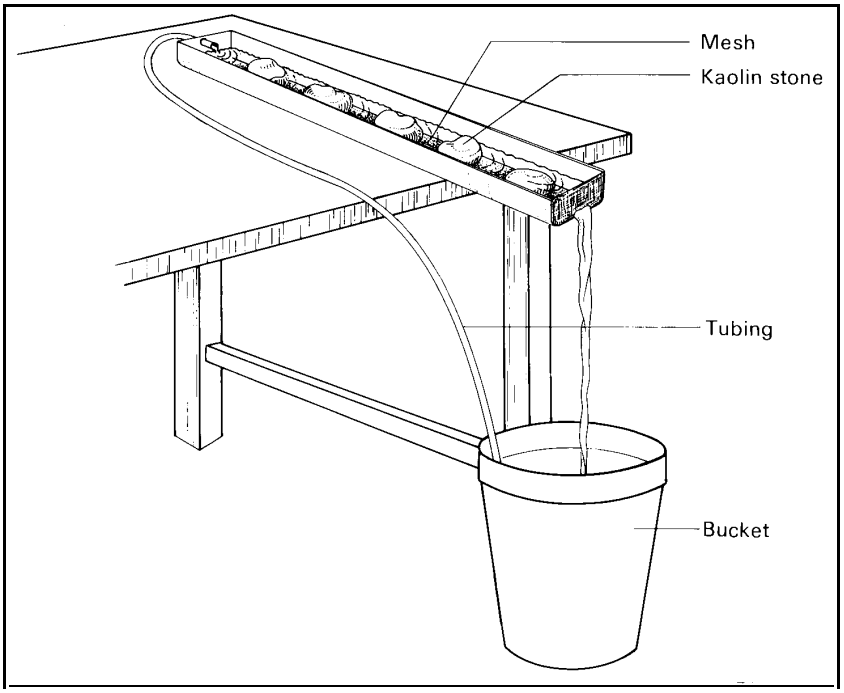

Figure 2.1 The artificial stream system, known as a *channel*, used during WET testing (DWAF, 2000).

The top-end of the channel was closed, with a stop-end containing a 12mm diameter hole through which 12mm diameter clear tubing was inserted. This tubing was attached to a submersible water pump to convey water from the bucket (or sump) to the channel. The "RENA Powerhead C20/C40" submersible water pump was placed in a plastic bucket filled with dilution medium, and recirculated the water around the system. At the other end of the channel, a screen (pore size 500 µm) allows water to pass, while preventing organisms from being washed out of the stream into the bucket. All joining parts of the channels were sealed with silicone glue to prevent

leaking. Four kaolin stones were placed in each stream as substrate, so that the organisms were not swept away by the water current. A small strip of gauze was also placed in each channel to serve as substrate.

A current was therefore maintained, thereby allowing organisms to irrigate their gills. This is particularly important for riffle-dwelling organisms, since a lack of flow of water causes decreased oxygen availability to the organisms (Lowell *et al*., 1995), and therefore stresses the organisms. This supports the recommendation that short-term toxicity tests with lotic organisms should be conducted under conditions of flow.

2.8 EXPERIMENTAL PROCEDURE

Before the start of the experiment, channels were checked for leaks, washed with tap water, acid washed with 5% hydrochloric acid solution, rinsed thoroughly twice with tap water, and allowed to dry. Pumps were also checked. Laboratory temperatures were controlled and stabilized before the start of the experiments and were checked daily. In the laboratory, about 15 litres of river water was poured into each 25 litres bucket and allowed to recirculate. The organisms were scooped with a jug from the cooler box and transferred on to a white tray, so as to be easily visible for sorting. Using a paintbrush or modified large-bore pasteur pipette, animals were transferred into channels after first being checked for missing limbs. Thirty to forty five organisms were used per channel (see Chapters 3 and 4 for details) to prevent over-crowding and to prevent conditions that may induce stress (APHA, 1992). Animals that were of similar size and life stage were used, as far as practically possible (APHA, 1992).

Organisms were allowed to acclimate to the laboratory conditions (Parrish, 1985; APHA, 1992), for 36 hours before effluent dilutions were added. Acclimation minimizes the probability that the measured responses will be due to factors other than the treatment. Mortality during acclimation should not be more than 10% (Buikema *et al.*, 1982; Parrish, 1985; APHA, 1992). If mortality is greater than 10%, the experiment should be repeated. In this study, acclimation mortalities were always well within the recommended limit. After the acclimation period, all dead organisms and exuviae were removed from each channel using a pasteur pipette. To minimize

handling of organisms, no attempt was made to try and standardize the number of organisms in the channels.

Effluent concentrations were prepared starting from the lowest percentage effluent concentration. 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 Jr. *et al*., 1996).

The test criterion was mortality, which was defined as immobility of the organism and lack of movement upon touching (Rand and Petrocelli, 1985; APHA, 1992). After the first hour each channel was checked and dead organisms were removed, counted, stored in 80% alcohol and labelled. During this process, the stream was left running to maintain constant flow. Torchlight was used to look for dead organisms in some cases, because of the dark colour of the effluent. Parameters such as electrical conductivity (EC), pH and temperature were also checked after the first hour, and daily thereafter. The values taken at the beginning of the experiment served as a guide to monitor any significant deviation in parameters as the experiment progressed. Mortalities were checked again after 3 and 5 hours of the first day. Screens were cleared with a fine brush to prevent clogging and pump intake sponges were also washed twice daily to reduce the accumulation of debris and faeces in the channels. After the first day mortalities were recorded twice daily. At the end of the test period, mortalities were recorded and all surviving animals were counted and preserved in 80% alcohol. The total experimental population comprised the total dead during the test, together with the total alive after 96 hours. 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. Exuvae were removed from the channels, and nymphs that had emerged and were found floating in the streams, were excluded when counting mortalities and calculating starting numbers.

The temperature, EC and pH of the channels were monitored daily. The temperature difference between the channels due to their positioning and uneven air circulation from air-conditioners was within the acceptable range of ±2°C (APHA, 1992). An

Amel digital conductivity meter (model 160, graphite electrode model 193) was used for EC measurements; pH readings were taken using a Checkmate CCA475627 kit (kraft effluent) or a Knicks climatic pH meter (textile effluent). Dissolved oxygen (DO) levels were not measured during the experiments as recirculation maintains consistently high DO concentrations (DWAF, 2000).

Any water loss due to evaporation and splashing was not replaced, as replacement would change the nominal concentration of the test solution. Modifying channel systems to reduce splash reduced water loss. When the experiment was completed, test solutions were diluted and discarded onto open grasslands (textile effluent) or open sandy areas (kraft effluent). Waste was not disposed into the sewer system, due to possible damage to the biological functioning of the system. All equipment was thoroughly washed with running tap water. A 5% HCl solution (APHA, 1992) was run through each stream, and then replaced with tap water for thorough rinsing.

To check if holding time was a factor in effluent toxicity, a sample of whole effluent was taken on the first day of the experiment, preserved using mercuric chloride ($HgCl_2$), and sent to IWQS for chemical analysis. Another sample from the same batch was allowed to stand in a tight-fitting container for 96 hours, thereafter preserved and also sent for analysis. Both samples were tested for the same parameters to see if there had been a change in the composition of effluent over the experimental period.

Water samples were taken from each channel at the beginning and end of each experiment, preserved with $HgCl_2$, and sent to the IWQS for full chemical analysis. IWQS has an accredited scientific laboratory. When the organisms in a channel reached 100% response during the course of the experiment, a water sample was immediately taken for chemical analysis. Methods of analyses are described in the DWAF Analytical Methods Manual (DWAF, 1992).

2.9 DATA PROCESSING

2.9.1 Statistical analysis of mortality data

Statistics are generally used to estimate the concentration of a toxicant that is sufficient to cause an effect. Several methods for the estimation of the LC50 and the associated confidence intervals are available. Parametric methods, e.g. Probit analysis, describe the concentration-response curve and therefore provide information such as the slope and effective concentration values from 1 to 99% response of the test population. Non-parametric methods, e.g. Spearman-Karber, are used when mortality distributions are not normal (Cooney, 1995). Both methods were used during this study.

Probit analysis

Probit analysis is a parametric statistical procedure for estimating the LC50 or EC50 values and the associated 95% confidence limits (Finney, 1971). It transforms concentration-response data to a known functional form before the LC50 is determined (APHA, 1992). Probit can only be used when at least two partial kills are present in the data set. The program for US EPA Probit calculations (Version 1.4) was used and follows the method described by Finney (1971).

A Chi-square (χ^2) test can be used to determine the adequacy of the Probit analysis model to the mortality data (Fowler and Cohen, 1993). The statistical significance of the chi-square heterogeneity can be read off chi-square distribution tables (df = n-2 where n is the number of concentrations used). If the chi-square value is very small, it indicates that there are insignificant differences between observed values and expected values. It also indicates that the model is appropriate and the LC50 may be calculated from the data (Buikema *et al.*, 1982). A large or significant chi-square value may indicate large random or systematic deviations of the observed values from the log-probit model (Buikema *et al.*, 1982).

Spearman-Karber and Trimmed Spearman-Karber

Spearman-Karber and Trimmed Spearman-Karber (TSK) methods are non-parametric procedures, which estimate the LC50 or EC50 and the associated 95% confidence

limits. These methods require only symmetry of the tolerance distribution around the LC50 (Hamilton *et al.*, 1977; APHA, 1992). Use of Spearman-Karber is recommended when partial mortality occurs, and the data do not fit the Probit model (US EPA, 1993). The disadvantage of using the Spearman-Karber method is that the test data must cover the range from 0 to 100% mortality (APHA, 1992). When monotonicity, i.e. response proportions consistently increasing with increasing concentrations, is not maintained, the TSK method smooths the data by averaging the points that define the line. The TSK method is a modification of the Spearman-Karber method, and is appropriate when the requirements for the Probit and Spearman-Karber methods are not met (US EPA, 1993). TSK eliminates the necessity for covering 0 and 100% response by trimming off the extreme values. The procedure therefore relies on the toxicity responses plotted along the linear portion of the concentration-response curve (Rand, 1995). In this study, LC50 values were determined for both kraft and textile effluents using TSK and Probit methods of analysis. In cases where less than 50% of the population was affected in the highest effluent concentrations, an LC50 value could not be calculated.

2.9.2 Graphical presentation of mortality data

Concentration-response curves and cumulative mortality curves

Raw data from an acute toxicity test are plotted as the proportion of mortality versus the exposure concentration at different observation times. This is known as a concentration-response curve, and usually shows that percentage mortality increases and then levels out as the toxicant concentration increases (Rand, 1995). The slope of the concentration-response curve indicates the sensitivity range of the organisms to the toxicant. The steeper the slope the sharper the threshold of effect, therefore the more intense the response over a narrow range of concentrations (Rand and Petrocelli, 1985). Log increases in toxicant concentration have been found to yield equivalent increases in response, but this function is toxicant dependent (Buikema *et al.*, 1982; Buikema and Voshell, 1993). When the concentration axis is plotted on a log scale, the idealized concentration-response curve is also a sigmoid curve, describing the tolerance levels of individuals in the population. When concentration-response data are transformed to a Probit scale, the concentration-response curve becomes a straight line (Buikema *et al.*, 1982; Rand, 1995). Cumulative mortality curves show

percentages of cumulative mortality plotted against time periods of the test at each effluent concentration.

2.9.3 Analysis and presentation of water quality data

Pollutants are normally presented to the test organism in a dilution medium, so both the pollutant and test organism are subject to the influence of the various aspects of the dilution-water chemistry. It is therefore critical to monitor general water quality conditions throughout a toxicity test as water quality can affect the chemical speciation of the toxicant and hence its bioavailability to the organisms (Pascoe and Edwards, 1989).

Ranges of physico-chemical constituents and nutrient concentrations were recorded and compared to water quality guidelines for ecosystem protection (see Chapters 3 and 4). Chemical analysis was conducted by the IWQS using standard methods (DWAF Analytical Methods Manual, 1992).

2.9.4 Hazard assessment

Toxicity test results have been used in this study to describe site-specific hazard-based guidelines for the effluents tested, which could be used in Ecological Risk Assessments (ERA). This approach, detailed in Palmer and Scherman (in press), should be considered preliminary. 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).

Palmer and Scherman (in press) have developed a method which relates toxicity test data (which describes the hazard), to resource classification. This method was applied to both kraft and textile mill WET test results. Each river reach can be classified in a

state of health ranging from natural (Class A), to highly modified with levels of water resource-use such as water abstraction and effluent disposal (Class D). If resource-use is causing ecosystem degradation beyond a sustainable level, the system may be classified as a Class E or F. These classes are defined by the "present state" condition of water quality, quantity, instream habitat, riparian habitat and the biota (Palmer, 1999). The class-related definitions for water quality and biota are given in Table 2.1 (modified from Palmer, 1999).

TABLE 2.1
WATER QUALITY AND BIOTIC CRITERIA WHICH MAY BE USED TO CLASSIFY AQUATIC ECOSYSTEM CONDITION IN SOUTH AFRICA (FROM PALMER, 1999)

Class A	
Water quality	Unmodified. Allow minimal risk to sensitive species. Remain within the target water quality range (TWQR, sensu DWAF, 1996f) for all constituents. No modification from reference conditions as defined by the rapid Bioassessment procedure SASS (South African Scoring System) (Chutter, 1994; 1998).
Class B	
Water quality	Use Aquatic Ecosystems guideline values (DWAF, 1996f) such as Chronic Effect Value (CEV) and TWQR to set objectives that pose a slight risk to intolerant organisms.
Biota	May be slightly modified from reference conditions. Especially intolerant biota may be reduced in numbers or extent of distribution.
Class C	
Water quality	Use Aquatic Ecosystems guideline values (DWAF, 1996f) such as Acute Effect Values (AEV), CEV, and TWQR to set objectives that allow moderate risk only to intolerant biota.
Biota	May be moderately modified from reference condition. Intolerant organisms may be absent from some locations.
Class D	
Water quality	Use Aquatic Ecosystem guideline values (AEV, CEV, TWQR) to set objectives that may result in high risk to intolerant biota.
Biota	May be highly modified from reference conditions. Intolerant biota unlikely To be present.

When toxicity results were analyzed using the EPA Probit programme, LC1, LC5 and LC50 values, with their 95% confidence limits, were generated. Each of these values (or toxicity test end-points) can be associated with a particular hazard description (Table 2.2; modified from Palmer and Scherman, in press). The toxicity test end-points were then ranked according to the percentage response, and then related to the resource classification system (Table 2.1; Palmer, 1999; Palmer and Scherman, in press). Table 2.2 shows selected end-points and associated hazard assessment descriptions (Palmer, 1999).

TABLE 2.2
EXAMPLES OF SELECTED TOXICITY END-POINTS AND ASSOCIATED HAZARD DESCRIPTIONS. SIMILAR DESCRIPTIONS CAN BE DERIVED FOR ANY LC VALUE OF THOSE LISTED HERE, THE LC50 IS THE MOST ACCURATE, AND THE LC5 AND LC1 INDICATE LOW HAZARD CONCENTRATIONS (PALMER, 1999)

Tolerance end-point	Hazard description
Below the low 95% confidence limit for the LC1	Concentrations at which there is a 95% probability that each nymph has <1% chance of mortality
Below the low 95% confidence limit for the LC5	Concentrations at which there is a 95% probability that each nymph has <5% chance of mortality
LC1	Best estimate of concentration where each nymph has a 1% chance of mortality
LC5	Best estimate of concentration where each nymph has a 5% chance of mortality
LC50	Best estimate of concentration where each nymph has a 50% chance of mortality

The AEV were calculated in each case according to DWAF (1996f), except that LC1 values for a single test species were used instead of the mean LC50 values of a wide range of species, as recommended in DWAF (1996f) and Roux *et al.* (1996). Table 2.3 shows an example of how to calculate the acute effect value using whole effluent acute toxicity results. LC1 values were used to calculate the AEV values. Chronic effect values were not calculated, as no chronic tests were conducted.

TABLE 2.3
EXAMPLE OF THE CALCULATION OF ACUTE EFFECT VALUE (AEV) USING WHOLE EFFLUENT TOXICITY TEST RESULTS FROM THE EXPOSURE OF THE MAYFLY *T. TINCTUS* TO GENERAL AND IRRIGATION KRAFT EFFLUENT

1.Acute Effects Value (AEV)

Step 1.
Calculate the Final Acute Value (FAV) – using the LC_1 for acute tests 4 Day exposure:
e.g. Experiment 1 (GKE)

LC1 = 3.8% effluent concentration

Step 2
2.Calculate the Acute Effects Value (AEV) where AEV = FAV/2

LC1 – based AEV = 1.9% effluent concentration

In this study, chronic testing was not undertaken, so the CEVs and chronic LC values were not available. However, chronic testing would be essential for a comprehensive set of hazard assessment guidelines. This method was applied to each batch of effluent, so that the percentage concentration of effluent with a particular chemical profile could be related to the instream hazard to the ecosystem. The mill manager could manage conservatively, as if it were the most toxic case in each instance. The application of this method is dealt with in detail in Chapters 3 and 4.

CHAPTER 3

EFFECTS OF KRAFT MILL EFFLUENTS ON NYMPHS OF THE MAYFLY *Tricorythus tinctus* Kimmins, FROM THE SABIE RIVER IN THE KRUGER NATIONAL PARK

3.1 INTRODUCTION

3.1.1 General

Pulp and paper plants are among the most polluting industries worldwide (Gökcay and Dilek, 1994). Pulp production from wood occurs at a rate of more than 10^6 tons per day world-wide, and about $200m^3$ wastewater per ton of pulp is discharged (Leuenberger *et al*., 1985). The latest available data on global pulp production are not published, but production is estimated at 171.5 million tons per year (SA Forest Owners Association, pers. comm.). Although the industrial conversion of wood into fibre and paper occurs primarily in the northern hemisphere (Owens, 1991), the pulp and paper industry is significant in South Africa (Steffen *et al*., 1990), particularly in Mpumalanga (DWAF, 1997b). South African production is 2.8 million tons per year, which is 1.63% of global production (SA Forest Owners Association, pers. comm.). Mpumalanga is home to the largest pulp and paper mill in South Africa, which uses the chemical process known as the kraft process, and is unique in that effluents are not discharged into a sewer or river system, but are irrigated, and reach the river system via percolation through the ground water.

Aquatic toxicology in general (Rand 1995; Walker *et al*., 1996), and specifically whole effluent toxicity (WET) testing (Grothe *et al*. 1996), are well established fields in the developed countries of the world, but are relatively new in South Africa (Palmer *et al*., 1996; Goetsch and Palmer, 1997; Slabbert *et al*., 1998a; Palmer and Scherman, in press). In this chapter the use of WET testing to evaluate the response of an indigenous riverine mayfly to different kraft mill effluents and to the receiving groundwater is reported.

In order to place this study in the context of both the WET testing approach and international trends in the management of pulp and paper effluents, this introductory section begins with a general description of kraft effluent, and goes on to detail the steps in the kraft process, with specific information about the Mpumalanga mill. A general review of investigations into the effects of pulp and paper effluents on aquatic environments follows. This highlights the northern hemisphere focus on the responses of fish to pulp and paper effluents. This also includes information on the responses of macro-invertebrates, algae and ecological processes.

3.1.2 Kraft effluent

General characteristics

The pulp and paper industry worldwide is recognised as one of the largest users of water. The volumes of water required for the kraft process results in considerable volumes of a wastewater. The wastewater is high in organic and inorganic material, leading to high Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Total Dissolved Solids (TDS) (Kovacs and Voss, 1992). High BOD is sustained, as there is often insufficient nitrogen to support degradation. Ammonia and BOD loads can be reduced with biological treatment such as activated sludge processes (Bryant *et al*., 1997). Waste discharge from kraft processing is typically coloured and contains high concentrations of Suspended Solids (SS) (Scrimgeour, 1989; Lagergren and Nystrom, 1991). The brown colour originates mainly from lignin degradation during the bleaching of pulp (Gökcay and Dilek, 1994). Colour in pulping effluents may reduce primary production by restricting light penetration, and therefore photosynthesis and secondary production by diminishing visibility. This can subsequently result in decreased feeding efficiency for organisms (Nawar and Doma, 1989; Owens, 1991). Fine bark and silt are the main cause of increased suspended solids in kraft effluent, which also contains toxic substances such as soaps of resin acids and sodium salts of unsaturated fatty acids (Kelso *et al*., 1977; Walden and Howard, 1977; Scrimgeour, 1989; Owens, 1991; Hakkari, 1992; Zender, *et al.*, 1994). These acids are relatively resistant to bio-degradation (Lagergren and Nystrom, 1991). Kraft effluent also contains inorganic nutrients, which cause eutrophication, and may stimulate

algal growth and microbial food supplies for invertebrates, e.g. nitrogen in the form of nitrate/nitrite, ammonia, and phosphorus (Lowell *et al*., 1995; Culp *et al*., 1996).

Effluent produced at the end of the whole process consists mainly of debarking wastewater, brownstock washing/screening water, sewered condensate and bleach plant filtrates (Servos, 1996). At the Mpumalanga mill there is no debarking effluent, and black liquor can only reach wastewater through leaks and accidental spills. The final product of the kraft process, bleached kraft mill effluent (BKME), is very acidic due to the reaction products during the bleaching process. This effluent is toxic and contains high amounts of organic matter (Nakamura *et al*., 1997). To meet regulatory requirements and/or to facilitate biological treatment of mill effluent, effluent can be neutralized, usually with calcium carbonate ($CaCO_3$) (Blackwell *et al*., 1989). Bleaching liquors are usually high in chloride content, which can cause problems for sensitive downstream users. An example is the irrigated tobacco fields downstream of the Mpumalanga mill, which has very stringent chloride requirements (DWAF 1996d). Organochlorines are released during the bleaching process when chlorine agents are used to remove residual lignin (Kelso *et al*., 1977; Owens, 1991). These compounds have the potential to induce long-term chronic toxicity to the environment at low sub-lethal concentrations (Axegard and Renberg, 1989 cited by Yetis *et al*., 1997).

The BKME is characterised by an unpleasant odour that is generated during the pulping process. Sulphur compounds have been identified in BKME, and these could cause the intense odour that persists in receiving waters (Brownlee *et al*., 1995). The odour is due mainly to the reduced sulphur gases, i.e. hydrogen sulphide, methyl mercaptan, dimethyl sulphide and dimethyl trisulphide (the most noxious) which may be reduced to dimethyl disulphide (Brownlee *et al*., 1995).

The manufacturing process

The production sequence for bleached pulp, newspaper and kraft linerboard is shown in Figure 3.1. It includes the following processes (SAPPI information leaflet):

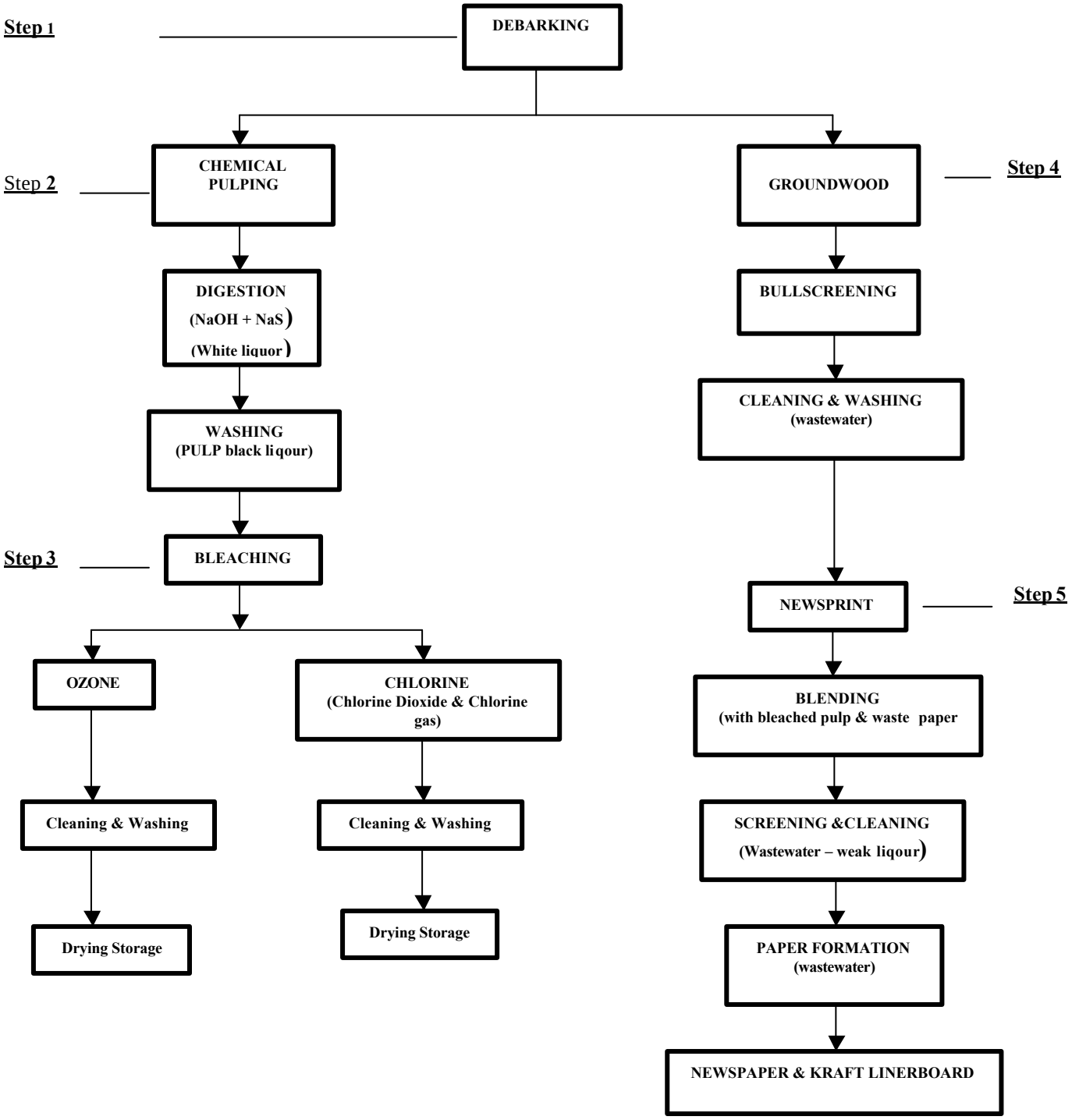

Figure 3.1 A flow chart of the pulp manufacturing process, including a common debarking stage (Step 1), followed by Kraft pulping (Step 2) and bleaching (Step 3); or a Groundwood process (Step 4) which leads to the Newsprint process (Step 5).

- Kraft pulping process;
- the groundwood processing; and
- newsprint processing.

In the first common step, logs are fed into a debarker for bark removal (Step 1, Figure 3.1). Debarked logs are conveyed to a chipper for kraft pulping process and to a sawmill for the groundwood process.

Kraft pulping process

1. Chemical Pulping (Step 2, Figure 3.1)

In the chipper, debarked logs are reduced to wood chips. These are conveyed to a chip bin, which feeds a pressurized steaming vessel. The steam, making the wood permeable to the cooling liquor, replaces air within the wood. Steamed chips are impregmented with cooling liquor. The chips are then cooked under pressure with white liquor (i.e. sodium hydroxide (NaOH) and sodium sulphide (NaS) solution). The lignin in the wood is dissolved, causing wood fibres to separate. These fibres are washed with water. Weak black liquor is produced. The crude pulp is screened and then sent to the bleaching plant.

Chemical pulping includes also a chemical recovery process, where black liquor from the digesters is sent to the evaporators, producing strong black liquor. From the evaporators, the condensate is taken to a recovery furnace where a smelt is formed from the chemicals. The smelt is dissolved to produce green liquor containing mainly NaS and $CaCO_3$. The green liquor is treated with lime and in the process white liquor is produced. The white liquor is then sent back to the digesters.

2. The Bleaching Process (Step 3, Figure 3.1)

The bleaching process is applied to the cooked pulp to remove any residual lignin, in order to brighten the finished product. This process occurs in stages. The unbleached pulp is sent to a three-stage diffusion washer where water moves counter-current to the pulp. The washed pulp is sent to a bleaching plant, which may use chlorine dioxide and/or ozone.

i) Chlorine Dioxide Plant

Chlorine dioxide and chlorine gas are added to the pulp before the bleaching stage. Thereafter it is washed with alkaline wash water to take the pH from 2.8 to 10-11. The wash water from this washing stage is sent to the effluent plant. HCl and NaOH are then used to adjust the pH to 4.2. Chlorine dioxide is again added to the pulp. The pulp is again washed and sent for storage where butrol and a chlorine scavenger are added. All the wash water is sent to the effluent plant.

ii) Ozone Plant

If ozone bleaching is followed, sulphuric acid is added in the storage tank to bring the pH down to about 3.2. From the tank the acidic pulp is sent to the ozone plant. The pulp is discharged into the Ozone Reactor for total ozone exposure. The mixed pulp is then washed with water, and wash water is extracted as effluent. The bleached pulp is then passed on for drying.

Groundwood process (Step 4, Figure 3.1)

From the sawmill the logs are ground and reduced to pulp. Pulp is washed, impurities removed and thickened. EDTA is then added to prevent discolouration formed by the presence of heavy metal ions in the wood. Excess water is drained away as wastewater. Part of this pulp is used in the manufacture of newsprint.

Newsprint process (Step 5, Figure 3.1)

The pulp from groundwood is blended with a portion of semi-bleached kraft pulp and waste paper from the newsprint machine. Water is added to create a 1% consistency as stock for the machine. The diluted pulp is then drained. The wet sheet of pulp then passes through presses to squeeze out the remaining water. Finally, a web of paper is formed and it undergoes various finishing steps to produce the desired paper.

3.1.3 Effects of pulp and pape r effluents on aquatic environments

The effects of pulp and paper effluents on aquatic environments have received considerable research attention in the past decade (reviews by Sodergren *et al.*, 1989; 1992; Servos *et al.*, 1996). Towards the end of the 1980s Swedish scientists drew attention to deleterious environmental effects associated with the discharge of BKME. They cited the effects on fish

which included changes in growth, maturation, mortality and recruitment leading to population shifts, as well as metabolic effects (Sodergren *et al*., 1989; Swanson, 1996). Smith and Sprague (1992) queried whether these same effects were apparent in North America. However, by 1996, Servos *et al*. (1996) could preface their substantial review of the environmental fate and effect of pulp and paper effluents, with the statement that the same kinds of effects had been associated with North American paper mills (Mc Master *et al*., 1991; Hodson *et al*., 1992; Munkittrick *et al*., 1992). A consideration of international approaches led to the following three questions, which underly the aims and approaches of this study:

- Why are pulp and paper effluents toxic?
- What are the environmental and biological effects that have been recorded?
- What are the end-points, which have been used to detect these effects?

Why are pulp and paper effluents toxic?

Once it was accepted that pulp and paper effluents had negative environmental consequences (Munkittrick *et al.*, 1992), organochlorines were the first components to be investigated as toxicants (Hakkari, 1992; Smith and Sprague, 1992; Sodergren, 1996). However, Smith and Sprague (1992), strongly supported by Robinson *et al*. (1994), found the organochlorines were not primarily responsible for toxicity to the aquatic environment. Although a considerable body of evidence confirmed this view (Sodergren *et al*., 1989; Kovacs *et al*., 1993; Ahtianen *et al*., 1996), there is evidence that unbleached, untreated effluents have environmental impacts (Smith and Sprague, 1992; Eklund *et al*., 1996; Verta *et al*., 1996). Fatty acids, resin acids, and sterols are now being implicated as the harmful constituents (Lagergren and Nystrom, 1991; Hakkari, 1992; Axegärd *et al*., 1993; La Fleur, 1996; Podemski and Culp, 1996; Stömberg *et al*., 1996). Effluent toxicity is also attributed to wood species and age (Kovacs and Ross, 1992; Verta *et al*., 1996). Leske (pers comm.) noted that effluent variability is associated with different types of trees. Excessive nutrient discharges could contribute to effluent toxicity or perhaps increase seasonal algal blooms in the receiving waters (Lowell *et* al., 1995; Culp and Podemski, 1996a; Bryant *et al*., 1997). However, "given the complexity and diversity of modern kraft mills and the transition state of the industry in response to environmental concerns, it is impossible to completely predict the chemical composition of effluents" (La Fleur, 1996),

and therefore it is very difficult to make causal links between environmental responses and specific effluent components.

What are the environmental and biological effects that have been recorded?

Many studies have described the biological effects of exposure to pulp and paper effluents. Examples from the many investigations of fish (review by Sandström, 1996) include:

- studies conducted at Jackfish Bay which found that fish exposed to primary-treated effluent from a bleached kraft pulp mill showed an increased age to maturity, smaller gonads, increased hepatic mixed-function oxygenase (MFO) activity, reduced fecundity with age in females and a reduction in male secondary sex characteristics (McMaster *et al.*,1991; Munkittrick *et al*., 1991, 1992, 1994, 1997; van der Heuvel *et al*., 1994);
- laboratory life-cycle tests using fathead minnows exposed to pulp mill effluents which confirmed depression in sex steriod production, delay in sexual maturity, reduction in egg production and changes in secondary sex characteristics (Robinson *et al*., 1994);
- tests showing elevated dioxin levels and changes in liver size, growth rate and gonadal size in fish collected downstream of bleached kraft mill (Munkittrick and van der Kraak, 1994);
- a study of goldfish which showed that depressions in the production of reproductive hormones occurred after exposure to effluent for as little as 4 days (McMaster *et al*., 1996);
- studies conducted near a bleached kraft mill discharge on the Pigeon River which found impacts such as decreased steroid hormone levels in sunfish (Adams *et al*., 1992);
- studies by Hall *et al.* (1991) and Soimasuo *et al.* (1998) which found that exposure to biologically treated bleached kraft effluent did not adversely affect growth and production of rainbow trout; secondary treatment of bleached kraft effluent substantially reduced the load of harmful constituents including fatty acids, resin acids and chlorinated phenolic compounds, and
- a study showing that chlorinated phenolics, resin acids and fatty acids may have inhibitory effects upon the growth and production of organisms exposed to effluents (Podemski and Culp, 1996).

The small numbers of investigations of macroinvertebrates and ecosystems processes indicated that pulp and paper effluents have been associated with increases in nutrients, with consequent increases in algal growth and changes in the feeding and food-web connections (Hall *et al*., 1991; Lowell e*t al*., 1995; Culp and Podemski, 1996a; Bryant *et al*., 1997). However, high concentrations of particulate and organic debris have also been shown to reduce light penetration, and therefore primary production. They also cause changes in filter-feeding and scraping functional groups (Mayack and Waterhouse, 1983).

In addition to these field studies, there are many traditional toxicity tests where whole organisms have been exposed to dilutions of BKME. In their recent review of the laboratory responses of whole organisms exposed to pulp and paper effluents, Kovacs and Megraw (1996) concluded:

- that they could find no general pattern relating new bleaching technologies to the chronic toxicity of final mill effluents;
- that pulping could be a source of residual toxicity;
- that there was still limited predictive capacity regarding aquatic ecosystem responses; and
- that the toxicological approach was a cost-effective means of assessing effluent quality.

What are the end-points, which have been used to detect these effects?

The end-points, which have been measured to assess the impacts of pulp and paper effluents, include:

- lethal and sub-lethal responses after acute or chronic exposure in standard toxicity tests (Kovacs and Megraw, 1996);
- whole organism tests which include life-cycle, partial life-cycle and early life stage assays (Sprague 1971; 1988); and
- the increasing use of biochemical and physiological end-points (reviews by Hodson, 1996; Lehtinen, 1996; and individual studies in Servos *et al*., 1996).

Less common are those studies which investigate the subtle responses of functioning ecosystems, with end-points such as changes in primary production (Lowell *et al*., 1995; Culp and Podemski, 1996b; Bryant *et al*., 1997) and feeding guilds (Mayack and Waterhouse, 1983). Biomarkers such as species diversity, reproduction and growth have been used as

indicators of environmental impact (Smith and Sprague, 1992).

Since this Chapter will ultimately focus on the responses of a riverine mayfly to concentrations of kraft mill effluent, and to groundwater samples, this consideration of the international pulp and paper industry focused mainly on reported biotic responses to kraft mill effluents.

3.1.4 Aims and approaches of the study

It has been daunting to approach the first toxicological study of the effects of kraft mill effluent on indigenous riverine organisms in South Africa. South African kraft mill technology is among the best in the world, with the option to use ozone rather than chlorination in bleaching (Steffen *et al*., 1990). In this study, *Tricorythus tinctus* from the Sabie River was used, as their responses to reference toxicants are known, and information is available on their tolerances to salts (Goetsch and Palmer, 1997; Palmer and Scherman, in press). The test animals were also abundant in the Sabie River; the availability of test organisms is crucial to toxicity testing using indigenous mayflies. The Elands and Sabie Rivers are in the same bioregion, i.e. the Lowveld, and are both characterised by rapids, riffles and marginal vegetation (Eekhout *et al*., 1996). Chemical analysis has also shown the water quality to be similar (Table 3.3). The Sabie River was therefore considered a suitable collecting site for test organisms.

This study aims to investigate the potential effects of kraft effluent on the indigenous riverine mayfly, *Tricorythus tinctus* Kimmins, from the Sabie River. It also reports on the relative toxicity of General and Irrigation Kraft Effluents (GKE and IKE) and groundwater surfacing as a spring downstream of the Mpumalanga kraft mill. The study also aims to provide a set of hazard-based guidelines, which relate effluent toxicity to river health. The effluent proved to be variable, so each effluent batch was characterized chemically, and specific percentage concentrations were related to a class of river health (DWAF, 1999). These data would allow mill managers to relate specific effluent chemistry, at specific concentrations, to the likely hazard they pose to in-stream river health. Since the kraft mill effluent is irrigated, and only reaches the in-stream environment via the groundwater, test organisms were exposed to groundwater over a

short-term chronic time period of twelve days. The study was conducted during two experimental periods:

- In two successive winter periods, weekly acute (96 hrs exposure) WET tests were conducted (1997, 1998), using GKE (1997 and 1998) and IKE (1998).
- One chronic (12 days) exposure to groundwater was conducted (1998).

3.2 PULP AND PAPER INDUSTRY IN SOUTH AFRICA

3.2.1 General

Timber growth, pulping and paper manufacture is predominantly a northern hemisphere phenomenon, with North America and Northern Europe producing 98 % of the world's paper. The global production of pulp and paper is about 440 million tons of which 4.8 million tons (approximately 1%) is the South African production (Table 3.1). Pulp and paper mills consume approximately 68.7% of timber produced. Pulp produces 76% (R6.9 billion) of total timber sales. The pulp and paper industry worldwide is recognized as the largest user of industrial water, and in South Africa it is the major producer of industrial wastewater (DWAF, 1996c). In South Africa, this industry consumes approximately 130 000 Ml of water per year (DWAF, 1996c). Compared to international pulp and paper industry standards, the South African pulp has much lower ratio of water per ton of product.

TABLE 3.1
SOUTH AFRICAN TIMBER CONSUMPTION AND PRODUCTION IN RELATION TO GLOBAL PRODUCTION; IMPORTS AND EXPORTS (1996/1997). (SA FOREST OWNERS ASSOCIATION, PERS. COMM.)

	Pulp	Paper & board	Total
Timber Consumption (million tons)	8.1	8.1	16.2
S A Production (million tons)	2.8	2	4.8
Global Production (million tons)	171.5	268.6	440.1
Exports (R Billion)	1.9 (33.8%)	2.1 (37.9%)	4 (71.7%)
Imports (R Billion)	0.14 (5.5%)	1.7 (69.8%)	1.84 (75.3%)

The South African pulp and paper industry dates back to 1920 when the Klipriver mill near Johannesburg started recycling waste paper into wrapping paper (Steffen *et al*., 1990). The first fully integrated chemical pulp and paper operation was started in 1938 using wheat straw as the basic raw material. However, the straw process was unsuccessful and in 1948, the mill changed to pulping of wood (Steffen *et al*., 1990). Since then, certain measures have been taken to reduce water use, and improve process and operation efficiency. The purpose of this is to help reduce the amount of effluent produced in the industry. Several methods of pulp production are employed in South Africa. The main types are chemical pulping, thermo-mechanical and mechanical pulping, of which the most extensively used in South Africa is a chemical process known as the kraft process (Steffen *et al.,* 1990). There are about 21 mills in South Africa ranging from small household tissue mills to the most modern integrated pulp and paper mills (Steffen *et al*., 1990). In countries like Canada, USA, Sweden and Finland, mill effluent is discharged into the receiving environment. In South Africa, wastewater is seldom disposed of directly to the river or sea with or without biological treatment, and at least two mills irrigate their effluents onto pastures.

The pulp and paper industry is a major contributor to the South African economy. Pulp and paper is injecting about R7 000 million per annum into the gross national product. The industry produces approximately 5 x 10^6 tonnes of pulp and paper products (in the form of newspaper, linerboard boxes, printing papers, tissues and package papers) annually (SA Forest Owners Association, pers. comm.). The South African pulp and paper industry imports approximately 70% of paper and 6% pulp, and exports approximately 38% paper and 34% pulp (Table 3.1).

As with most industrial enterprises there are environmental costs. Pulp and paper production impacts heavily on catchments. The area of timber plantation in South Africa is estimated at 1.5 million ha (57% pine, 35% eucalyptus and 8% wattle) covering 1.2% of South Africa (Scott *et al*., 1998). Timber growing places significant demands on the available water resource, reducing mean annual flow stream by 3.2% and low flows by 7.8% (Scott *et al*., 1998), thus affecting the water balance of the affected catchments.

The type of wood used and the pulping process produces a complex effluent with numerous compounds such as organochlorines from bleaching, some of which have been found to be toxic (Owens, 1991). Organochlorine compounds such as polychlorinated biphenyls (PCBs) have demonstrated environmental persistence, The type of wood used and the pulping process produces a complex effluent with numerous compounds such as organochlorines from bleaching, some of which have been found to be toxic (Owens, 1991). Organochlorine compounds such as polychlorinated biphenyls (PCBs) have demonstrated environmental persistence, long-range environmental transport, and either bioaccumulation from receiving waters or biomagnification through the food web (Owens, 1991). When pulp and paper effluent is discharged into the river, it introduces fibre and suspended solids, organics and nutrient enrichment; it causes colour changes and increases turbidity of the receiving water. All these parameters cause adverse environmental impacts. This problem seems avoidable through the use of irrigation as the soil acts as a filter. However, chlorides remain a residual problem as they pass through the soil and may increase chloride levels in groundwater.

3.2.2 Case study: The Mpumalanga kraft mill

The Mpumalanga mill is situated at the confluence of the Ngodwana and Elands Rivers approximately 50 km from Nelspruit. Pine and eucalyptus are the two main trees used for the production of pulp. Wood consumption averages 6000 tons daily. The mill water usage is approximately 35 Ml, and production is about 27 Ml of combined effluents daily. These effluents undergo primary treatment, and are then used to irrigate 514 hectares of kikuyu grass pastures.

The primary treatment consists of pH adjustment (using green liquor dregs generated in the chemical recovery process), followed by sedimentation, to remove suspended material. The effluent plant has two holding dams with a total capacity of 37 Ml, one acts as a storage dam for irrigation and the other is kept empty at all times for emergencies. The effluent contains contaminants such as lignin derivatives, chlorides, sodium and sulphate. Sodium is readily adsorbed onto soil particles, while chloride ions pass through the soil structure and into the Elands River via groundwater, which surfaces at three Dolomite springs on the northern side of the irrigation fields. The mill produces two types of effluent, namely, general and bleaching effluent. The general mill effluent consists of effluent from the waste plant, kraft linerboard, newsprint, groundwood, and pulp plants. This effluent is relatively low in TDS and chlorides, and is degradable. The bleaching effluent consists of effluent from bleaching stages, the chlorine dioxide and demineralization plants (SAPPI conf. doc.).

The general effluent is sent to a clarifier and the overflow is sent to an irrigation sump and then pumped to irrigation dams. The bleaching effluent is sent to its own clarifier, the overflow is sent to the irrigation sump and then pumped to the irrigation dams. The underflow from both clarifiers is sent to a dewatering press where water is recycled back to the effluent sump. The treated effluent is then transferred into one of the two irrigation dams. Gypsum ($CaSO_4$) is added to the effluent to balance the pH before it is irrigated.

Groundwater (X-EYE)

Groundwater in the area between the irrigation fields and the receiving Elands River, surfaces at several dolomitic "eyes" or springs, making it more than usually accessible. Mill records were scrutinized, and the X-EYE was selected as the source water for tolerance testing since it showed the highest TDS and EC levels. Groundwater is one of the routes for the movement of chemical constituents from non-point sources to the downstream receiving body. It is mainly groundwater from perched and deep aquifers that transports dissolved constituents. Groundwater slowly accumulates the different ions or salts that have been dissolved or leached out from the surrounding rocks. Therefore, it often has higher salinity and hardness compared with surface waters. The interaction between water and rocks allows some ions to be taken up into solution.

During irrigation, some wastewater does percolate through the soil and downward through cracks and fissures in the rock material (DWAF, 1995). Long-term accumulation of ions from wastewater and those dissolved in groundwater may lead to gradual enrichment of the groundwater. If specific ion concentrations reach high levels, they can lead to water quality problems and impact on different water users and the riverine biota (DWAF, 1995). The chemistry of the groundwater is presented in Section 3.5.1.

Pollution of groundwater remains long after the contaminants have entered the groundwater system. This is due to the slow movement of the pollutants through the groundwater system. Diffuse, as opposed to point source pollution, is particularly problematic as it can affect an entire aquifer and can go unnoticed for decades (DWAF, 1991). Since the kraft process effluents are irrigated, and reach the river via the groundwater, groundwater quality management is an important aspect of the mill's environmental management program. Because of downstream tobacco farming, DWAF has imposed a limit of 20 mg/l for chlorides in the Elands/Crocodile River system (SAPPI conf. doc).

3.3 STUDY SITE

The Mpumalanga mill chosen as the effluent supplier for this study is adjacent to the Elands River, which is the receiving water environment that can be impacted by any mishap from the mill (Figure 3.2). The Sabie River is where test water and test organisms were collected from (Figure 3.3). The test organisms were abundant in the Sabie River and the their availability is crucial to toxicity testing using indigenous mayflies. Chemical analysis has also shown the water quality to be similar.

3.3.1 Elands River

Description of Elands River

The Elands River is the major tributary of the Crocodile River and rises in a gently sloping Highveld zone near the town of the Machadodorp. Further downstream, the pulp and paper mill is situated at the confluence of the Ngodwana and Elands Rivers. The section of the river from Waterval-Boven to Ngodwana is characterised by exceptional riffle and rapid habitats. The waterfall at Waterval-Boven is an outstanding geomorphological feature of this river reach, and forms a natural barrier to the upstream migration of fish. Trout farming is of economic and recreational importance to the people of the Machadodorp area.

In 1989, a spill of kraft effluent took place in the Elands Rivers. A survey conducted two days after the spill indicated that large fish mortalities had occurred downstream from the confluence of Ngodwana and Elands Rivers, and up to the confluence of the Elands and Crocodile Rivers. Virtually all fish in this segment were destroyed (Kleynhans *et al*., 1992). A survey during 1991 indicated recolonization of the affected area (James and Barber, 1991). Fish populations downstream of the mill had not yet recovered two years after the effluent spill, but at a site immediately downstream the mill below the confluence of the Elands and Ngodwana Rivers, species-rich populations and healthy fish were present. Upstream of the mill, the fish fauna was found to be relatively diverse and abundant.

Vegetation

A narrow belt of near-forest occurs along the riverbanks. *Acacia robusta, Albida, Breonadia salicina* and *Euphorbia* species were identified. Much of the natural vegetation has been impacted by farm dams and bank erosion, and has been replaced by exotic plantation (Engelbrecht and Deacon, 1998). However, the area just before the confluence of the Crocodile River is characterised by mature riparian trees, and shrubs in excellent condition.

Water quality

Water quality in the Elands River appears to be good, with all parameters low except for phosphorus in the form of ortho-phosphate (Table 3.3). Downstream in the Elands River, high electrical conductivity and chlorine concentrations are due to the presence of a paper mill (Heath and Claassen, 1999). Kleynhans (1999) found the water quality and habitat conditions of the segment of the Elands River that was impacted by the 1989 spill, to have improved considerably since the spill.

3.3.2 Sabie River

Topography

The catchment covers about 709 600 ha. The river arises on the eastern escarpment and flows through more than 74 000 ha of commercial forestry plantations (pine trees and eucalyptus). It reaches its confluence with the Sand River some 125km to the east, inside the western boundary of the Kruger National Park (KNP), passing through the south-central sector of the southern part of the KNP (Weeks *et al*., 1997).

The Sabie River comprises the main stem of the Sabie/Sand River System, with the Sand and Marite Rivers acting as major tributaries. It remains one of the few South African perennial, unregulated rivers and is the least impacted of the six rivers traversing the KNP (Venter and Deacon, 1995; Weeks *et al*., 1997). It has a mean annual rainfall (MAR) run-off of 762 mm; 91.2% of which originates in the eastern escarpment and foothill regions, which are the headwaters of the catchment. The flow varies seasonally

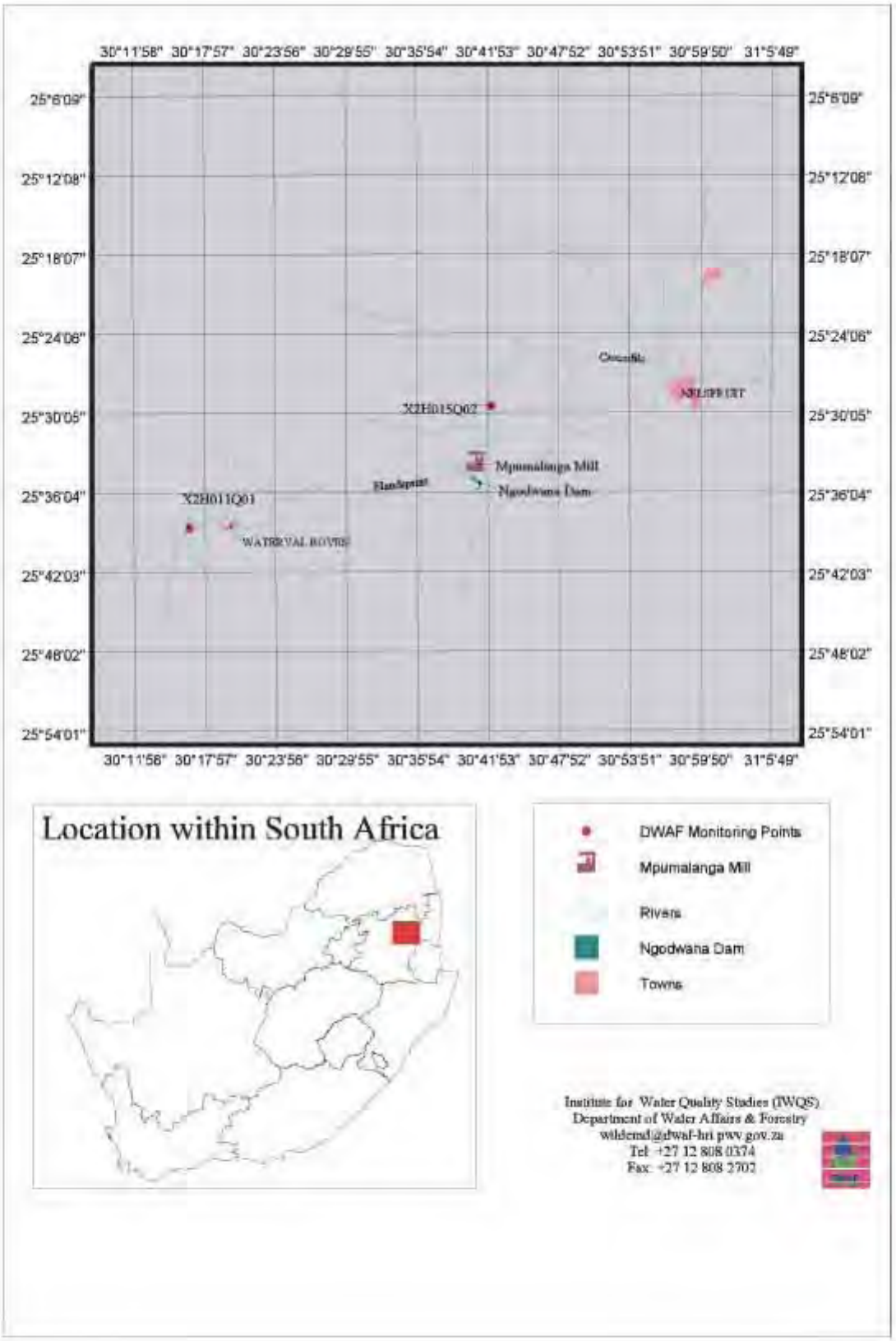

Figure 3.2 Map of Elands River showing the Mpumalanga mill and DWAF water quality site Geluk (X2H011Q01).

with summer peaks (February) and low flows at the end of the season (October) (Weeks *et al*., 1997). The region is also subject to unpredictable tropical cyclones and drought. Water temperatures vary with altitude and given the steep gradient of the system, there are considerable changes from the Middleveld to the Lowveld regions.

Vegetation

The riparian strip is lined by pine and gum trees and surrounded by afforestation. Vegetation bordering the stream is predominately herbaceous, with grasses and trailing roots predominating. In some areas riparian trees dominate the banks, with reeds occurring in open areas (Weeks *et al.*, 1997).

Land use

The major water consumers of the Sabie River are irrigation farming and extensive afforestation (mainly pine and eucalyptus). The rapidly growing population is placing an increasing demand upon the river for domestic consumption and cattle farming. To meet the demand, the DWAF have identified several potential sites for impoundment in the Sabie/Sand system. Small mining enterprises also take place in the towns of Sabie and Graskop.

Water quality

Water quality in the Sabie River is generally considered to be very good and is reflected in the high biotic diversity which is characteristic of the river (O'Keeffe *et al*., 1989). Turbidity is low during low flows and occasionally increases during high flow spates (Gore *et al*., 1992). Salinity values (EC) are relatively low, ranging from 4 to 37 mS/m (O'Keeffe and Davies, 1991). pH is within the neutral range, but the water is poorly buffered and is sensitive to change. There is a trend of progressive increase in TDS down the river. Total dissolved solids are mainly due to natural causes, with an increase in sodium, chloride and sulphate and a corresponding decrease in calcium, magnesium and total alkalinity (O'Keeffe and Davies, 1991). Nutrients concentrations are generally low except during drought periods (Weeks *et al.*, 1997).

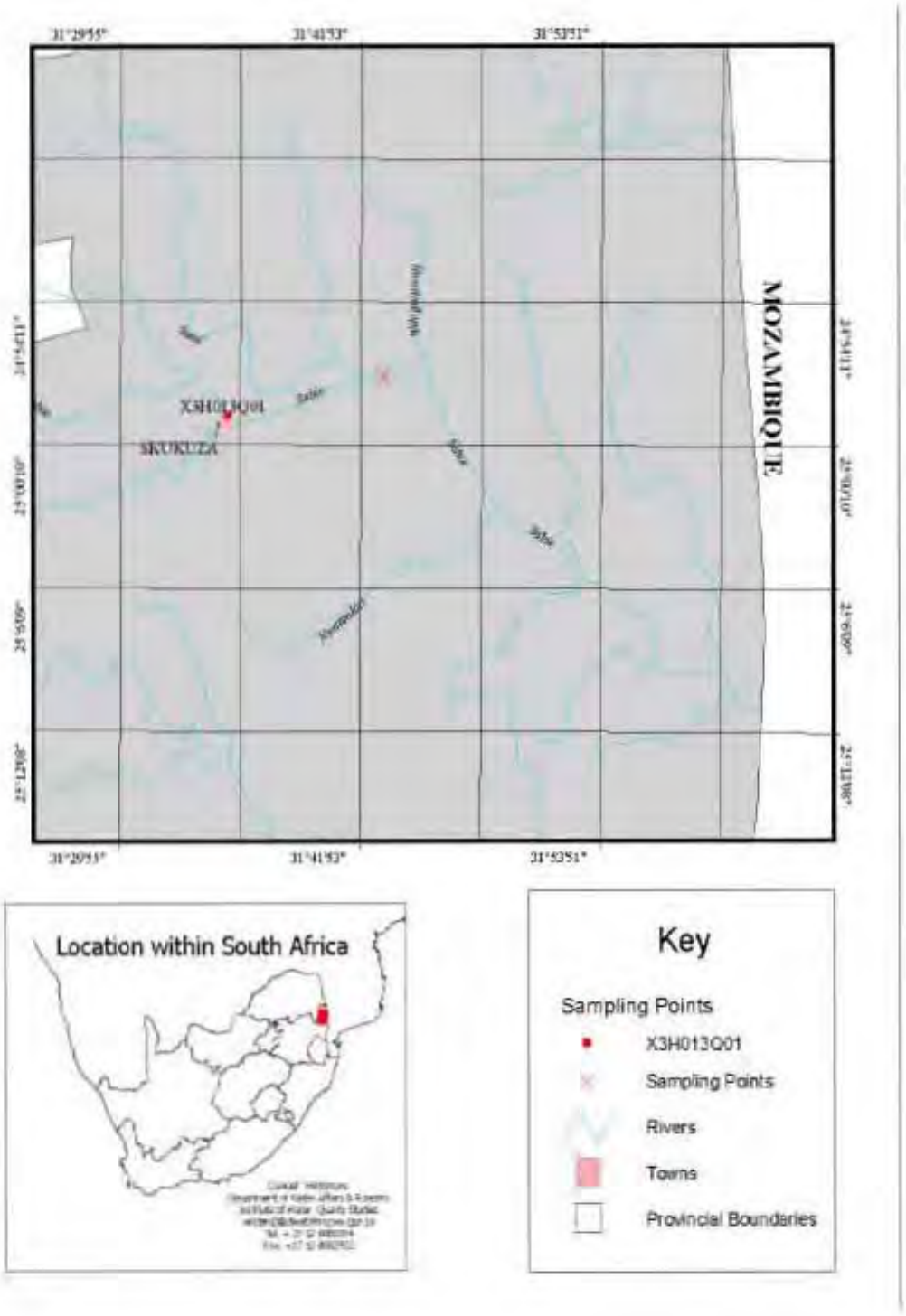

Figure 3.3 The sites in the Sabie River that were used during the study for the collection of water (X3H013Q01) and test organisms (X) respectively.

3.4 MATERIALS AND METHODS

The methods used are detailed in Chapter 2, and only those issues specific to the kraft mill effluent experiments in the KNP are mentioned in this section.

3.4.1 Collection of test organisms and experimental medium

T. tinctus is riffle-dwelling invertebrate species, and test organisms were collected from the Sabie River about 3 km below the High Water Bridge (Figure 3.3). The collection site is comprised of a cobble-riffle reach. Organisms were abundant on the underside of the stones. *T. tinctus*, like *T. discolor*, is a filter feeder, with filtering setae on the mouthparts (Palmer *et al.*, 1993a, b). It is indigenous to both Sabie and Elands Rivers, and generally forms a considerable proportion of the communities (Kleynhans, 1999).

Sabie River water was collected from the weir outflow (DWAF water quality monitoring point X3H013Q01) (Figure 3.3) near Skukuza, on the day of the experiment. It was used as the test diluent and as the control medium. A weir outflow was chosen because the water is always fast flowing, constantly aerated and relatively sediment-free.

The kraft effluent and groundwater were collected as grab samples from the paper mill in 25 litre plastic containers. The general effluent (GKE) was collected from one of the five channels that direct effluent away from the factory to the clarifiers. The irrigation effluent (IKE) was collected from the outlet of the pipeline, as it is discharged into the holding dam before it is irrigated. Irrigation effluent is the combined effluent from the clarifiers. This effluent is treated with $CaSO_4$ to adjust the pH before being discharged into the dams. The groundwater was collected as surfaced spring water from the site termed X-EYE. The effluent and groundwater were couriered from the paper mill to Skukuza, and effluent was never kept for more than 24 hrs before being used in an experiment (APHA, 1992).

3.4.2 Experimental approach

Laboratory design

Experiments were conducted in an artificial stream laboratory equipped by the Kruger National Park Rivers Research Programme (KNPRP), at Skukuza, Mpumalanga. Laboratory temperature was maintained between 17°C and 22°C with the use of two air-conditioners. Lighting was maintained at a 12:12 hour light:dark cycle with OSRAM biolux tubes providing wavelength of light similar to that of sunlight (Palmer *et al*., 1996; DWAF, 2000).

Experimental stream systems and experimental procedure

The channel systems used for toxicity tests, and the experimental procedures are described in Chapter 2. After an acclimation period of 36 hours, the 96 hr acute toxicity tests were conducted, using Sabie River water as the diluent and control water, kraft effluent as the toxicant, and *T. tinctus* as test organisms. Test organisms were exposed in artificial stream channels, to increasing percentage kraft effluent concentrations in a regression design (Table 3.2). One short-term (12 day) chronic experiment was run,exposing test organisms to mixtures of surfaced groundwater collected from X-EYE and Sabie River water. Since groundwater elicited a low chronic response neither Probit nor Trimmed Spearman-Karber analyses could be undertaken and a simple percentage response was recorded (Slabbert, pers comm.).

TABLE 3.2
PERCENTAGE CONCENTRATIONS OF KRAFT EFFLUENT USED FOR ACUTE (96 HRS) AND GROUNDWATER MIXTURES FOR CHRONIC (12 DAY) TOXICITY TESTING WITH SABIE RIVER WATER AS DILUENT AND CONTROL.

Experiment number	Type of effluent	Effluent concentration (%)	Starting date
1	General Kraft Effluent (GKE)	0.5,1,2,3,4,5,10,30,50	06-08-1997
2		10,15,20,25,30,40,50	13-08-1997
3		1,3,10,30,40,50,75,100	20-08-1997
4		1,3,10,30,40,50,75,100	27-08-1997
5		1,3,10,30,50,100	13-08-1998
6		1,3,10,30,50,100	20-08-1998
7	Irrigation Kraft Effluent (IKE)	1,3,10,30,50,75,100	17-08-1998
8		1,3,10,30,50,75,100	17-08-1998
9		1,3,10,30,50,75,100	20-08-1998
10		1,3,10,30,50,75,100	20-08-1998
11	Groundwater	10,25,50,100	29-07-1998
12		10,25,50,100	29-07-1998

Water quality analysis

1997

In the winter of 1997, four batches of GKE were used. The whole effluent was chemically analysed by the IWQS at the start and finish of the each experiment. Routine daily measurements of pH, EC and temperature were taken in each experimental channel. An Amel digital conductivity meter (model 160, graphite electrode model 193) was used for EC measurements and a Checkmate CCA475627 kit for pH reading. Water samples were taken at the start and finish of each experiment for nutrient analyses (ammonium, nitrate, nitrite and phosphate) in the Skukuza laboratory, using a Merck Spectroquant 118 photometer, following Merck Manual Photometer SQ118 Methods.

1998

In the winter of 1998, two batches of GKE, and four batches of IKE were used. Since the degree of effluent variability had become obvious in the previous year, samples were sent from

each channel for full analysis by IWQS at the start and finish of each experiment. Therefore nutrient analysis was not undertaken in the Skukuza laboratory, and only routine daily parameters (pH, EC, temperature) were measured.

Data analysis

The experiments were set up using a regression design with one channel at each concentration, plus a control. The Trimmed Spearman-Karber and EPA Probit methods were used as described in Chapter 2, to calculate the acute (96 hrs/4d) LC50 values (with upper and lower 95% confidence limits). Where the EPA Probit and the Trimmed Spearman-Karber method gave similar results, the Probit result was used since this method provided LC1 and LC5 values. The acute LC1 values were calculated from 1997 and 1998 data, and were used to derive the Acute Effect Value (AEV) (Section 1.2.3). The AEV, LC1, LC5 and associated confidence limits were then used to apply the hazard-based approach of Palmer and Scherman (in press), as described in Chapter 2 (Section 2.9.4). This approach links toxicity test results to river health classification (DWAF, 1999a; Kleynhans, 1999).

3.5 RESULTS

In this study, attention was paid to the toxicity of two kraft effluents: (i) GKE and (ii) IKE. A total of ten acute (96 hours) toxicity tests were conducted on kraft effluent. Two short-term chronic (12 day) tests were also performed with mayfly nymphs exposed to groundwater. The Elands and Sabie River comparative water chemistry data are presented. Chemical analysis of effluent was undertaken as it provides an indication of effluent composition and variability. Effluent samples were analyzed by the IWQS (DWAF, 1992).

3.5.1 Chemical composition of Elands and Sabie River waters, kraft effluent and groundwater

Tables 3.3 and 3.4 present ranges of selected physico-chemical constituents and nutrient concentration analysis data profiles of Elands River and Sabie River water, kraft effluent (GKE, IKE) and groundwater, respectively. Elands River data was taken from the DWAF

TABLE 3.3
PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENT CONCENT RATIONS (EXPRESSED IN MG/L) OF THE ELANDS RIVER (1998), AND THE SABIE RIVER (1997 AND1998). VALUES GIVEN INDICATE THE RANGES FROM ALL WATER SAMPLES COLLECTED. THE ELANDS RIVER DATA INDICATE RANGES FROM DWAF SAMPLING STATION X2H011Q01 (GELUK) (1998).

Parameter (mg/l)	Elands River	Sabie River
EC (mS/m)	10.3-15.3	13.0 – 15.8
TDS	121.0-127.0	96.0 - 119.0
pH	7.9-8.2	7.9 - 8.0
TAL	71.0-86.0	59.0-64.0
Na^+	4.0-6.0	6.0 - 7.0
K^+	0.6-1.5	0.9
SO_4^{2-}	5.0-12.0	4.0-10.0
Cl^-	4.0-6.0	10.0
Mg^+	10.0-12.0	6.0 - 7.0
Ca^{2+}	5.0-12.0	9.0 - 10.0
NH_4^+-N	<0.04-0.09	<0.04 - 0.5
NO_3^-+NO_2^- -N	<0.04-0.08	<0.04 - 0.2
PO_4^{3-} -P	0.01-0.05	<0.005

(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 are for samples collected from 1997 to 1998, and IKE data from the 1998 samples. Groundwater data are for samples collected in 1998 from a dolomite spring, X-EYE, between the irrigated fields and the Elands River.

TABLE 3.4

COMPARATIVE PHYSICO-CHEMICAL CONSTITUENTS OF KRAFT EFFLUENT (EXPRESSED IN MG/L). MPUMALANGA MILL SAMPLES COLLECTED IN WINTER FROM: THE GENERAL EFFLUENT STREAM–GKE (1997 AND 1998), THE IRRIGATED EFFLUENT STREAM-IKE (1998), AND THE GROUNDWATER EMERGING FROM A DOLOMITE SPRING, X-EYE (1998) -BETWEEN THE IRRIGATED FIELDS AND THE ELANDS RIVER, FOR ACUTE AND CHRONIC (12 DAY) TOXICITY TESTING. DATA ARE PRESENTED AS RANGES AND WERE ANALYZED BY IWQS.

Parameter (mg/l)	General Effluent (GKE)	Irrigation Effluent (IKE)	Groundwater (X -EYE)
EC (mS/m)	151.0 – 408.0	252.0- 395.0	109.0-143.0
TDS	1181.0- 2286.0	1904.0 - 2536.0	820.0-837.0
pH	6.9 - 8.3	6.5- 6.9	8.3
SO_4^{2-}	259.0 – 974.0	401.0 - 465.0	158.0-166.0
TAL	<4.0-222.0	0.4 - 1.4	<0.05
Cl^-	45.0- 79.0	630.0 - 897.0	256.0-264.0
Ca^{2+}	48.0- 203.0	58.0 - 61.0	70.0-76.0
K^+	19.5 – 51.1	2.9 - 32.7	0.9-1.2
Na^+	164.0 – 1115.0	564.0 - 776.0	100.0-105.0
NH_4^+-N	<0.04 – 0.10	0.1- 0.2	<0.05
$NO_3^- + NO_2^-$ -N	<0.04- 0.10	<0.04 - 0.10	0.5-1.1
PO_4^{3-}– P	0.0	0.4-1.4	0.0
Mg^{2+}	12.0 – 33.0	23.0 - 37.0	65.0-75.0
Cr-soluble	<0.005	<0.005	<0.005
Cu-soluble	<0.005	<0.005	<0.005
Fe-soluble	0.9	8.7	<0.005
Zn-soluble	0.1- 0.8	0.1	<0.05
Al-soluble	0.2 - 4.6	1.2	<0.02
B-soluble	0.4 - 1.2	0.2	<0.005

As shown in Table 3.4, salts are a major component of the effluent, particularly sodium and sulphate ions. TDS and EC are higher than in either river (Table 3.3) with sodium, sulphate, chloride and calcium ions being the major contributory factors (SRK Inc, 1990; Dallas and Day, 1993). In some experiments, the pH of GKE was higher than 10.0 (alkaline). The IKE showed high levels of chloride (Cl), sulphate (SO_4^{2-}), and sodium (Na^+). The pH of irrigated effluent was lower than the GKE indicating the presence of bleaching effluent. This effluent is characterized by low pH due to acids used during the

process. Except for dissolved Al and Zn, nutrients and trace metals were generally below detectable levels.

3.5.2 Comparison of Probit and Trimmed Spearman-Karber (TSK) LC50 values

Table 3.5 presents a comparison of Probit and TSK mortality data of the different kraft experiments. In this study, most of the experiments yielded similar data, with overlapping 95% confidence limits. The groundwater results could not be analysed 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). For Experiments 8 and 9, Probit analysis was not appropriate for the data.

TABLE 3.5
LC50 VALUES AND 95% CONFIDENCE LIMITS, FOR *T. TINCTUS*, IN KRAFT EFFLUENT. 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; c^2=CHI SQUARE).

Experiment number	Type of Effluent	Probit				Trimmed Spearman-Karber			
		LC50	95% LCL	95% UCL	c^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	General Kraft Effluent (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
11	Ground-Water	PROBIT NOT APPROPRIATE				TSK NOT APPROPRIATE			
12						88	71	110	42

3.5.3 Toxicity test results and associated effluent chemistry

Experiments were analyzed individually, and are graphically presented as percentage cumulative mortality and concentration-response curves (Figures 3.4 - 3.23). The daily measurements of individual experiments monitored during the toxicity testing are also presented in Tables 3.6 - 3.15. Full physico-chemical results for all the kraft experiments are presented in Appendix A, Tables A1-A8. Appendix B lists all Probit and Trimmed Spearman-Karber data.

Experiments conducted in 1997

Experiment 1 General Kraft Effluent (06-08-1997)

Description: *GKE Exp 1*

Exposure of *T. tinctus* to GKE for 96 hours was conducted at a range of effluent concentrations (1-50%). A 100% effluent concentration was not included due to excessive foaming of the effluent. The responses to this batch of effluent are shown in Figures 3.4 and 3.5. Full physico-chemical analysis is presented in Appendix A, Table A1.

Water quality

Ranges of values for daily measured variables and nutrient levels of GKE are shown in Table 3.6. The 50% effluent concentration had only one reading, as test organisms died within the first 3 hours.

This batch of effluent was characterized by a blue-black colour, and high levels of turbidity due to a high concentration of Suspended Solids (SS). High SS reduce light penetration, decrease primary production and food availability to organisms, and mayclog filter-feeding setae (Dallas and Day, 1993). These could therefore be contributory factors to mortality. The temperature within each channel fluctuated between 16 and 18.5°C, varying by about 1 to 2°C over the 96 hour test period. The pH in the channels was stable, fluctuating by ±1unit, and was within the recommended range, pH 6.5-9.0, for guidelines for the protection of aquatic ecosystems (DWAF, 1996f). In effluent concentrations above 5%,

the nutrient levels showed an increase as effluent

TABLE 3.6
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 1. * INDICATES THAT ONLY ONE READING WAS TAKEN AS 100% MORTALITY WAS OBSERVED WITHIN THE FIRST 3 HRS. (ND = Not Done)

Para-meter (mg/l)	% Effluent concentration									
	Control	**0.5**	**1**	**2**	**3**	**4**	**5**	**10**	**30**	**50**
EC (mS/m)	10.4-13.7	11.0-13.8	13.3-17.3	16.9-19.8	21.1-23.8	20.0-27.9	28.9-33.0	47.7-53.6	121.0-133.5	189*
PH	6.8-8.1	7.1-8.1	7.1-7.6	7.0-8.1	7.4-8.6	7.1-7.5	7.2-8.1	7.3-7.6	7.9-9.7	ND
Temp. (°C)	16.0-17.0	16.0-18.0	16.0-17.3	16.0-18.0	16.5-18.0	16.0-17.8	16.5-18.0	16.0-18.0	17.0-18.5	18*
NH_4^+-N	<0.04-0.05	<0. 04	<0.04-0.01	<0.04-0.02	<0.04-0.04	<0.04-0.05	0.02-0.06	0.05-0.09	0.2	ND
NO_2^-	0.03-0.04	0.03-0.05	0.08-0.09	0.07-0.1	0.09-0.1	0.13-0.2	0.15-0.2	0.26-0.28	0.56	ND
NO_3^-	0.9-1.1	2.1-3.1	1.9-3.0	4.2-5.2	5.0-5.4	6.2-6.9	9.1-10.7	12.4	28.0-61.6	ND
PO_4^{3-}	<0.01	<0.01-0.02	<0.01-0.2	<0.01-0.1	0.1-0.15	0.17-0.2	0.18-0.2	0.46-0.52	1.69-1.22	ND

concentrations increased. Nitrate levels were much higher than 10 mg/l, which is the nitrate General Effluent Standard for South African conditions (DWAF, 1991).

Total Dissolved Solids (TDS) fluctuated between 2286 and 2412 mg/l, and EC ranged from 408-420 mS/m, almost 20 times that of the receiving water and the sampling site (Appendix A, Table A1). Chloride levels were low and the range was 45-70 mg/l. Sodium concentrations recorded were also high at 1115 mg/l, and more than 100 times that of the sampling site. The maximum sulphate level recorded was 1086 mg/l, which is about 100 times that of the sampling site (10 mg/l), but lower than the TWQR set at 1 400 mg/l (DWAF, 1996f).

Toxicity results

Mortality in the control channel was zero, indicating good quality of water and good health of organisms. Effluent concentrations up to 4.0% were the similar to the control, with zero

mortality. Figure 3.4 showed only 50% effluent concentration reached 100% mortality. The concentration-response curves (Figure 3.5) showed that at 50% effluent concentration all of the organisms died in the first 12 hrs. The Probit LC50 was calculated at 19% effluent concentration, with a narrow range of confidence limits (Table 3.5), and a Chi-square heterogeneity of 7.5. Trimmed Spearman-Karber (TSK) analysis was also conducted and the LC50 was 20% effluent concentration, with confidence limits similar to that of Probit. The percentage trim was zero, indicating the suitability of the model.

Experiment 2 General Kraft Effluent (13-08-1997)

Description: *GKE Exp 2*

T. tinctus was exposed to GKE for 96 hours at a range of effluent concentrations (1-50%). Different effluent concentrations were chosen in this experiment, as Experiment 1 showed little response to very low effluent concentrations. This batch of effluent was quite different from the first batch of effluent. There was no foaming and the colour was brownish. The responses to this effluent are shown in Figures 3.6 and 3.7. Full chemical analysis is shown in Appendix A, Table A1.

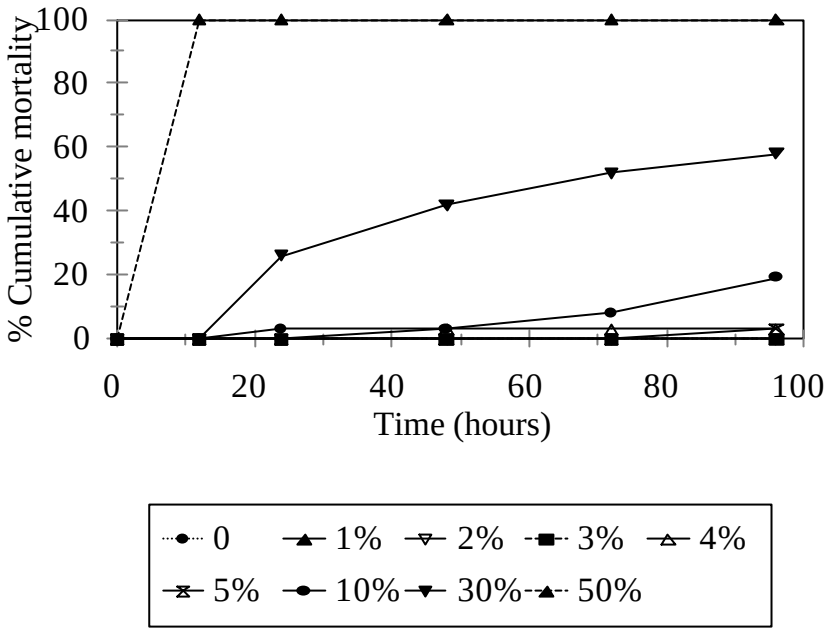

Figure 3.4 Experiment 1: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to General Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water. The zero mortalities of concentrations in the 0-5% range cannot be distinguished along the x-axis.

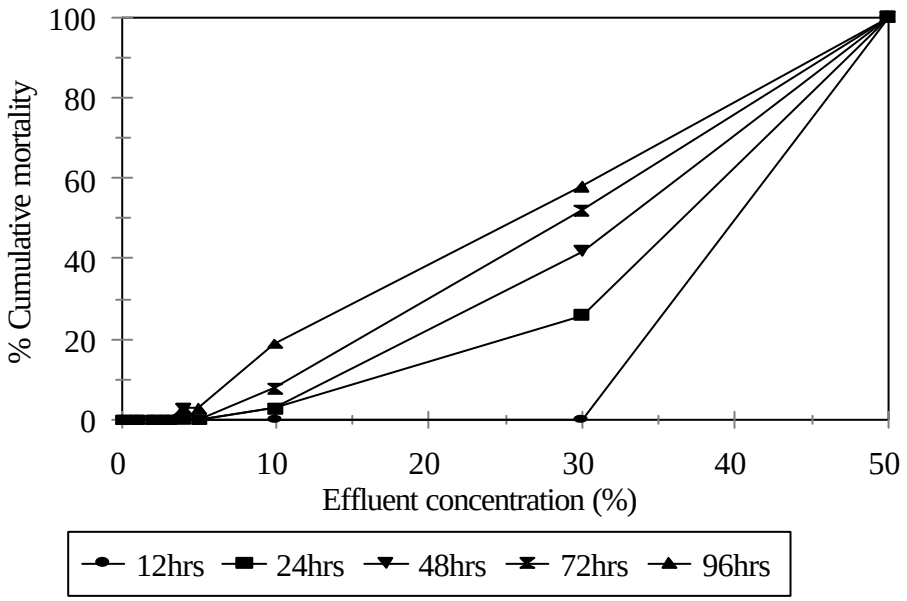

Figure 3.5 Experiment 1: Concentration-response curve for *T. tinctus* exposed to General Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

Water quality

Table 3.7 presents the range of values for daily measured variables and nutrient levels during Experiment 2.

TABLE 3.7
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 2

Para-meter (mg/l)	% Effluent concentration								
	Control	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**50**
EC (mS/m)	9.9-10.8	20.1-21.0	25.6-26.7	30.0-31.4	35.2-36.7	40.2-42.0	44.7-46.7	49.6-52.1	50.0-61.4
pH	7.0-7.6	7.0-7.3	6.7-7.3	6.8-7.1	6.8-7.1	6.9-7.1	6.9-7.2	6.9-7.1	6.9-7.7
Temp. (°C)	15.0-18.0	15.8-18.0	15.8-18.0	16.0-19.0	16.0-18.5	16.0-18.5	16.0-19.0	16.3-19.0	16.0-18.5
NH_4^+-N	0.05-0.3	0.05-0.2	<0.05-0.04	0.42-0.66	0.39-0.45	0.51-0.53	0.48-0.87	0.28-0.55	0.46-0.82
NO_2^-	0.03-0.05	0.09-0.17	0.11-0.14	0.12-0.16	0.13-0.18	0.14-0.19	0.14-0.22	0.13-0.24	0.15-0.32
NO_3^-	0.0-1.9	3.0-3.4	1.1-3.6	3.1-3.9	2.4-4.0	7.1-21.6	8.3-18.0	4.0-7.4	7.9-44.0
PO_4^{3-}	0.04-0.28	0.01-0.42	0.01-0.26	0.02-0.37	0.02-0.41	0.02-0.41	0.03-0.49	0.04-0.58	0.05-0.85

This batch of effluent was characterized by lower physico-chemical parameters as compared to Experiment 1. The pH in the channels was stable, fluctuating by less than 1.0 pH unit (Table 3.7). Temperatures within each channel varied by ±3°C, which is within the recommended ± 3°C (DWAF, 2000), over the 96 hr test period (Table 3.7). Nutrient levels and trace metals were low, i.e. within the General Standards, except for nitrate levels, which were also high in Experiment 1. TDS and EC were both lower, about half the values in Experiment1. Sulphate and sodium levels were much higher than the sampling site (Appendix A). This effluent sample appeared to be the least saline of the effluents used.

Toxicity results

At 50% effluent concentration, which was the highest concentration, mortality was 77% after 96 hrs and control mortality was 3% (Figure 3.6). The concentration-response curves showed that half of the organisms died at 32% effluent concentration, after 96 hrs (Figure 3.7). The Probit LC50 was calculated at 30% effluent concentration (Table 3.5). This was the first indication of the degree of variability of the effluent, which showed moderate toxicity compared with Experiment 1. The chemical variables of this effluent were comparable to the irrigation effluent in Experiments 7 and 10 (Appendix B).

Experiment 3 General Kraft Effluent (20-08-1997)

Description: *GKE Exp 3*

Exposure of *T. tinctus* to GKE for 96 hrs was conducted in the full range of effluent concentrations (1-100%). The use of different effluent concentration ranges in Experiment 1 and 2 did not give a clear indication of effluent toxicity, and it was apparent that 1-100 % effluent concentrations should always be used. Acclimation mortality was 3%. Figure 3.8 shows the percentage cumulative mortality and Figure 3.9 shows the concentration-response curve during the acute (96 hrs) toxicity testing.

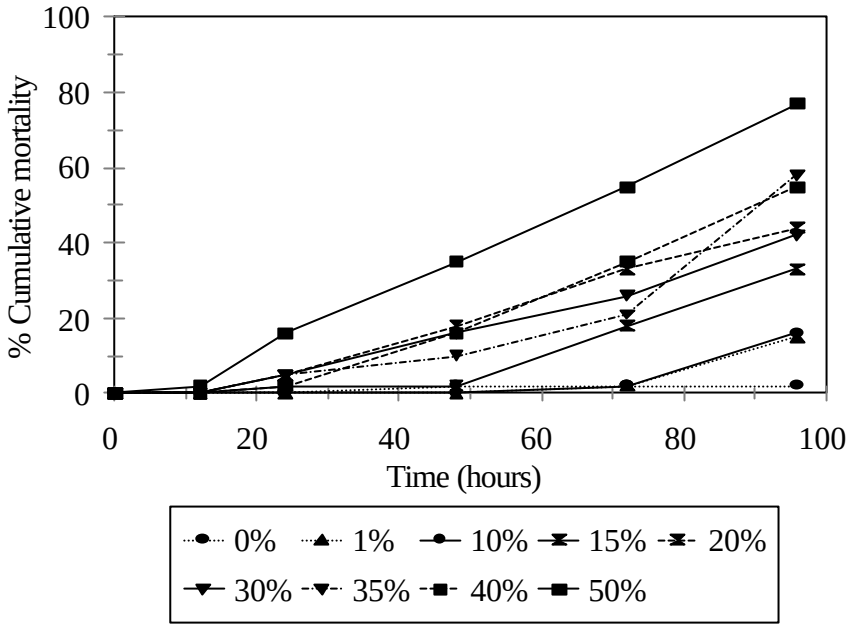

Figure 3.6 Experiment 2: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to General Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water. The zero mortalities of 1% concentration cannot be distinguished along the 0% response axis.

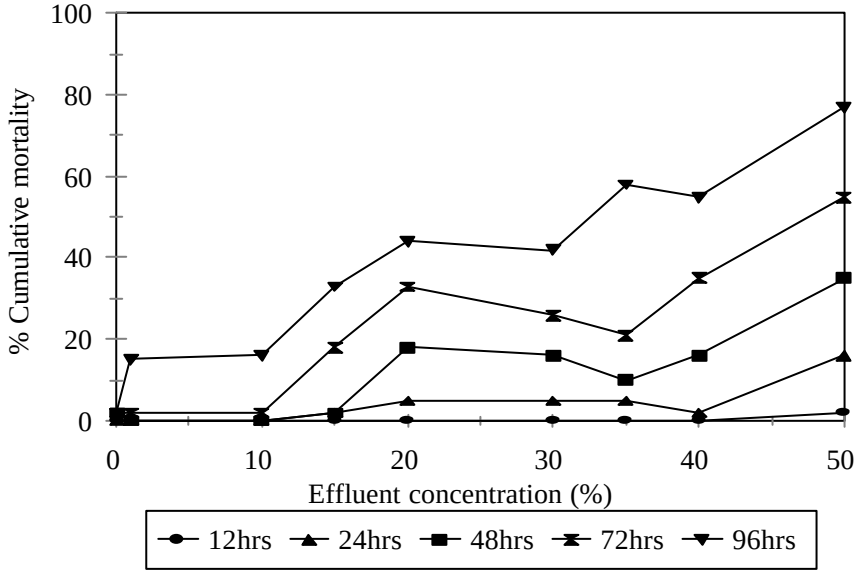

Figure 3.7 Experiment 2: Concentration-response curve for *T.tinctus* exposed to a range of General Kraft Effluent for various time periods (12-96 hrs). The diluent was Sabie River water.

Water quality

Table 3.8 presents the range of values for daily measured variables and nutrient levels during Experiment 3.

TABLE 3.8
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 3. * SHOWS THAT ONLY ONE READING TAKEN, AS 100% MORTALITY WAS REACHED WITHIN 2 HRS OF THE EXPERIMENT. ND= NOT DONE

Para-meter (mg/l)	% Effluent concentration								
	Control	**1**	**3**	**10**	**30**	**40**	**50**	**75**	**100**
EC (mS/m)	10.3-11.3	13.1-14.1	19.1-19.9	39.5-41.1	95.2-100.5	132.4*	110.6*	130.8*	167.8*
pH	7.6-7.9	7.4-7.6	7.0-7.4	7.1-8.9	7.8-9.7	9.9*	9.9*	9.9*	10*
Temp (°C)	17.5-18.0	17.5-18.0	18.0-19.0	18.5-19.5	18.5-19.0	19*	ND	ND	ND
NH_4^+-N	0.04-0.28	0.0-0.19	0.0-0.27	0.0-0.14	0.14	0.18*	22*	31*	51*
NO_2^-	0.02-0.08	0.05-0.1	0.08-0.09	0.18-0.19	0.5*	1*	0.8*	1.3*	1.6*
NO_3^-	0.0-1.3	2.9-8.7	4.2-8.5	12.9-13.6	63*	95.5	108	127	129.8
PO_4^{3-}	0.01-0.04	0.04-0.09	0.26-0.1	0.32-0.53	1.5*	2*	2.3*	3.4*	4.6*

This batch of effluent was more alkaline, with pH in the channels ranging between 7.0 and 10.0. The temperatures in each channel varied by less than 1.0° C. Nitrate levels were generally high than the recommended standard of 10.0 mg/l (Table 3.8). Phosphate levels were exceptionally higher than 1.0 mg/l, which is the standard (Appendix A). TDS were much higher than the other GKE experiments, and about three times that of Experiment 2 (Appendix A). EC was high, but less than that in Experiment 1. Sodium levels were higher than the sampling site and contributed to high TDS of the effluent. Sodium is involved in ionic, osmotic and water balance in all organisms (Dallas and Day, 1993). Sulphate levels (average 628 mg/l) were more than sixty times that of the sampling site concentrations (Table A1). Excess amount of sulphate may form sulphuric acid that reduces the pH

of the water. This can have severe impact on aquatic ecosystem (Dallas and Day, 1993). This effluent was among the most saline of the effluents tested in this study.

Toxicity results

Mortality was 100% within the first 12 hours in 40, 50, 75 and 100% effluent concentrations. After 12 hours, 30% effluent concentration had more than 80% response, and there was very little response in 0 to 10% effluent concentrations. Mortality for 3% effluent concentration was less (6%) than in the control after 96 hrs. By the end of the experiment (96 hrs), all concentrations above 10% showed 100% mortality, while 10% effluent concentration showed 18% mortality (Figure 3.8). The responses observed in this batch of effluent were totally different from the previous two experiments. The concentration-response curve was very steep between 10 and 20% effluent concentration (Figure 3.9), suggesting an intense response over a narrow range of concentrations. The Probit LC50 was calculated at 9% effluent concentration with wide confidence limits. The TSK LC50 was set at 14% effluent concentration, with a narrow range of confidence limits and zero % trim (Table 3.5).

Experiment 4 General Kraft Effluent (2-08-1997)

Description: *GKE Exp 4*

The responses to this batch of GKE are shown in Figures 3.10 and 3.11. Acclimation mortality was zero percent. Full physico-chemical analysis is presented in Appendix A, Table A1.

Water quality

Table 3.9 presents the range of values for daily measured variables and nutrient levels during Experiment 4.

The pH range was normal in lower effluent concentrations, but wide and high in higher effluent concentrations. The temperatures within the channels fluctuated between 16.5 to 19.5°C, varying by ±2°C over the test period (Table 3.9). Nutrient levels were generally low,

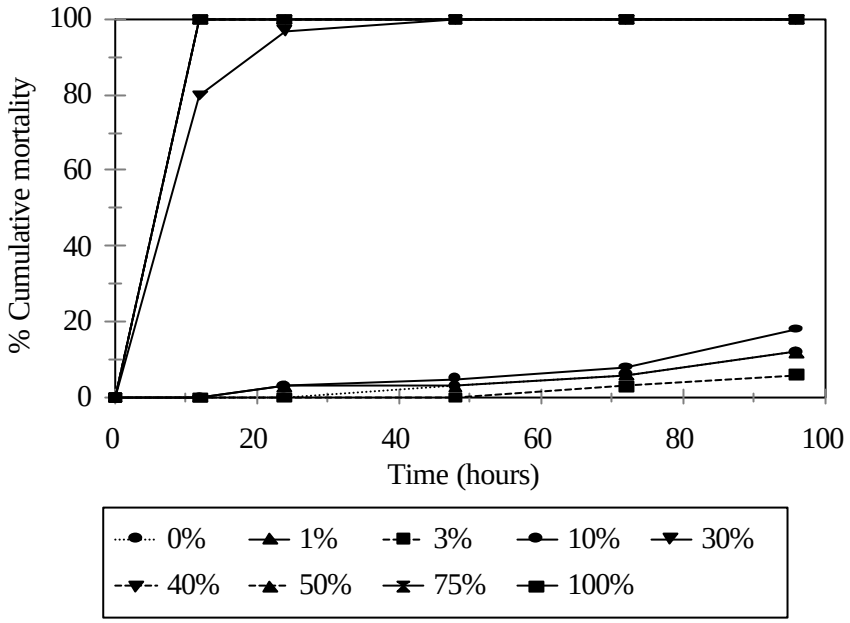

Figure 3.8 Experiment 3: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to General Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water.

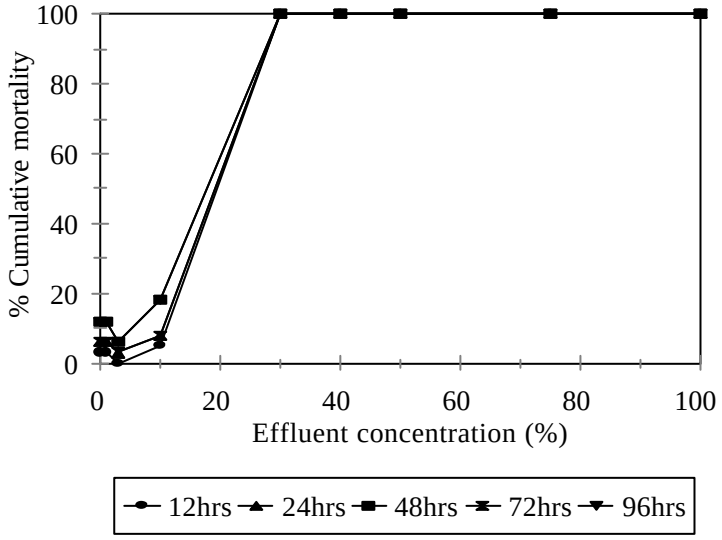

Figure 3.9 Experiment 3: Concentration-response curve for *T. tinctus* exposed to General Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

with the exception of nitrates. Nitrates increased with increasing concentrations .

TABLE 3.9
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 4.

Para-meter (mg/l)	% Effluent concentration								
	Control	**1**	**3**	**10**	**30**	**40**	**50**	**75**	**100**
EC (mS/m)	10.8-11.7	11.2-12.5	12.7-13.7	21.9-22.9	36.2-37.2	40.2-42.1	62.3-67.1	88.8-99.0	111.5-128.5
pH	6.9-7.8	6.8-7.5	6.8-7.5	6.8-7.3	6.9-7.0	7.1-9.4	7.3-9.9	7.6-10.4	7.6-10.8
Temp. (°C)	17.0-19.0	17.0-19.0	17.0-18.5	16.5-19.0	18.5-19.0	18.5-19.5	17.8-19.0	19.0	18.5-19.5
NH_4^+-N	0.0-0.24	0.0-0.02	0.26-0.40	0.28-0.80	0.82-3.45	0.95-3.6	0.0	0.0-0.1	0.0-0.1
NO_2^-	0.04-0.08	0.02-0.1	0.03-0.08	0.09-0.11	0.13-0.17	0.20-0.11	0.23-0.27	0.36-0.41	0.47-0.49
NO_3^-	0.0-1.9	0.0-3.7	0.0-7.5	1.0-8.5	3.5-8.4	3.0-12.4	11.1-12.9	7.7-18.6	5.3-23.0
PO_4^{3-}	0.01-0.03	0.01-0.03	0.04-0.06	0.02-0.26	0.09-0.61	0.07-0.78	0.35-1.06	0.87-1.66	1.26-2.14

Toxicity results

In this experiment, the responses appeared to increase gradually with time and according to effluent concentrations. In the first 12 hrs, the 50% effluent concentration had higher mortalities than the 75 and 100% effluent concentrations (Figure 3.10). The first 100% mortality occurred in the 100% effluent concentration after 24 hrs, followed by the 50 after 72 hrs and 75% effluent concentration after 48 hrs (Figure 3.10). The response pattern was again different from other experiments. After 96 hrs, the 30 and 40% effluent concentrations were close at 95% mortality. The 10% effluent concentration showed 48% mortality, while 3% effluent concentration had the same response as Experiment 3 at 18%. The concentration-response curve showed that at 50% effluent concentration all of the organisms died after 72hrs while at 10% effluent concentration, half of the organisms died by 96 hrs

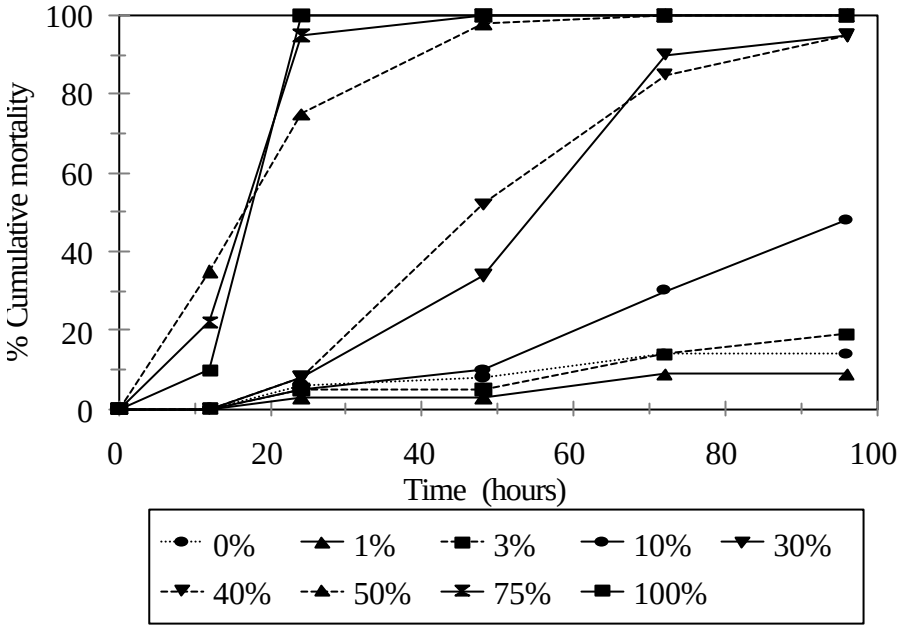

Figure 3.10 Experiment 4: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to General Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water.

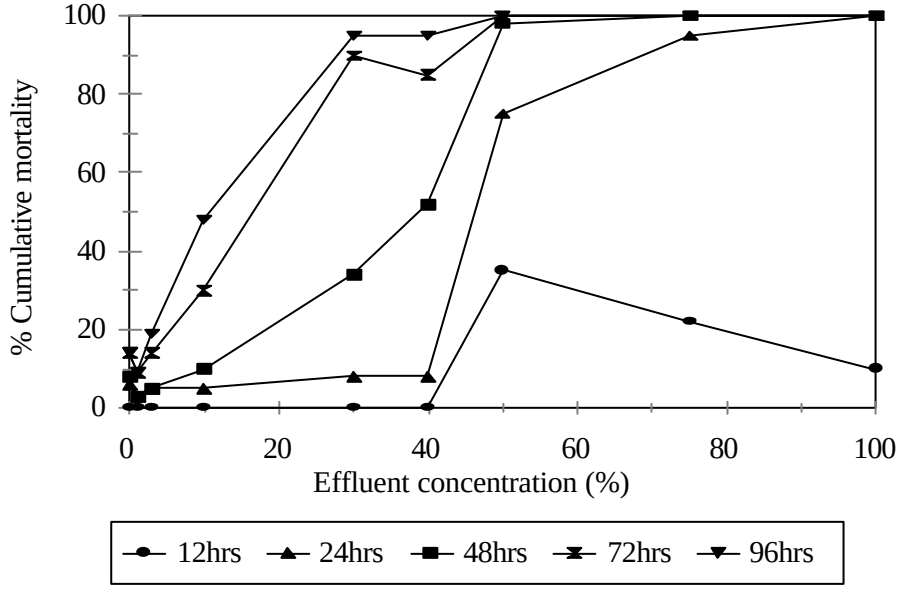

Figure 3.11 Experiment 4: Concentration-response curve for *T. tinctus* exposed to General Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

(Figure 3.11). The Probit LC50 was calculated at 12% effluent concentration (Table 3.5). The effluent toxicity is comparable to that of Experiment 3.

General comment on 1997 experiments

The various batches of effluents were chemically totally different from each other (Appendix A), although all samples were collected from one site, but at different times. In Experiment 1, sulphate, sodium, EC and TDS were much higher than the other three experiments. Chlorides were generally low, as expected, as the effluent is not mixed with bleaching effluent, which is known to contain organochlorines. In Experiments 1, 2, 3 and 4, trace metals, iron, aluminium and manganese were higher than those of Experiments 6 and 7, which were also GKE (Appendix A). The addition of effluent to the diluent increased the colour in varying degrees as the effluent concentration increased.

In 1998, the general kraft effluent experiments were repeated and irrigation kraft effluent was investigated. The same toxicity testing procedure was followed. Problems were encountered during the running of some experiments. High mortalities were observed in one set of channels due to a power failure experienced during the experiment. The affected channel's results were discarded. In one instance, suspended solids were so high that the channels became clogged at high effluent concentrations. A "window" was cut at the top of the end mesh to alleviate this problem. Nutrients were not analyzed in the laboratory, as the previous experiments did not show significant changes. However, full chemical analysis for all the concentrations was conducted for each experiment by the IWQS.

Experiments conducted in 1998

Experiment 5 General Kraft Effluent (13-08-1998)

Description: *GKE Exp 5*

T. tinctus was exposed to GKE for 96 hrs at the full range of effluent concentrations (1-100%). Figure 3.12 shows the cumulative effect of the kraft effluent over the 96 hrs, and Figure 3.13

depicts the concentration-response relationship over 96 hrs. A full chemical analysis of individual concentrations in presented in Appendix A, Table A3.

Water quality

Table 3.10 presents ranges of daily measured variables during Experiment 5. Nutrients were not monitored in the laboratory, as the results of Experiments 1-4 showed no significant differences between the experiments.

TABLE 3.10
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 5. * INDICATES THAT ONLY ONE READING WAS TAKEN.

Para-meter	% Effluent concentration						
	Control	**1**	**3**	**10**	**30**	**50**	**100**
EC (mS/m)	11.2-13.2	11.9-14.5	13.2-18.0	17.5-27.3	30.2-55.1	43.4-82.2	75.2*
PH	7.4-7.8	7.5-7.9	7.5-7.8	7.5-7.9	7.3-8.0	7.1-8.0	5.7*
Temp. (°C)	15.0-19.5	15.0-19	14.5-18.0	15.0-18.5	15.0-19.0	14.5-18.5	19.0*

This batch of effluent was characterised by a brownish colour and a high concentration of suspended solids. The pH for the effluent was low (5.7). The pH in the diluted concentrations ranged between 7.1 and 8.0 (Table 3.10). The temperatures within the channels fluctuated between 14.5 and 19.0°C, varying by ±4°C. Increased water temperature could affect the ionic and osmotic balance of aquatic organisms (Dallas and Day, 1993), and the amount of oxygen that dissolves in the water (Rand and Petrocelli, 1985). EC, TDS, Na^+, SO_4^{2-} and Cl were lower than other experiments (Appendix A, Table A3), indicating that this effluent is less saline. Nutrient concentration levels were also low.

Toxicity results

In this experiment, 100% mortality was reached in the 100% effluent concentration within the first 5 hours (Figure 3.12). After 96 hrs, the 50% effluent concentration response was

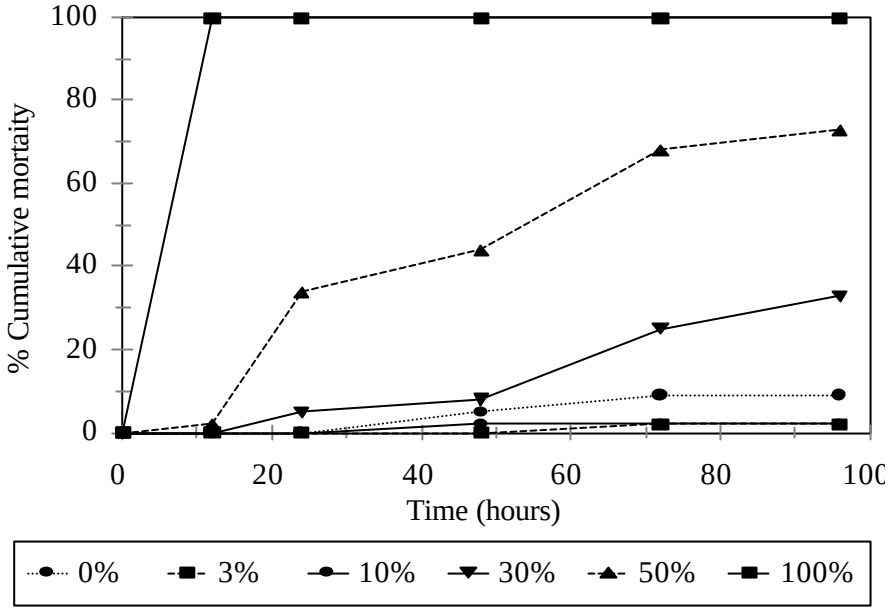

Figure 3.12 Experiment 5: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to General Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water.

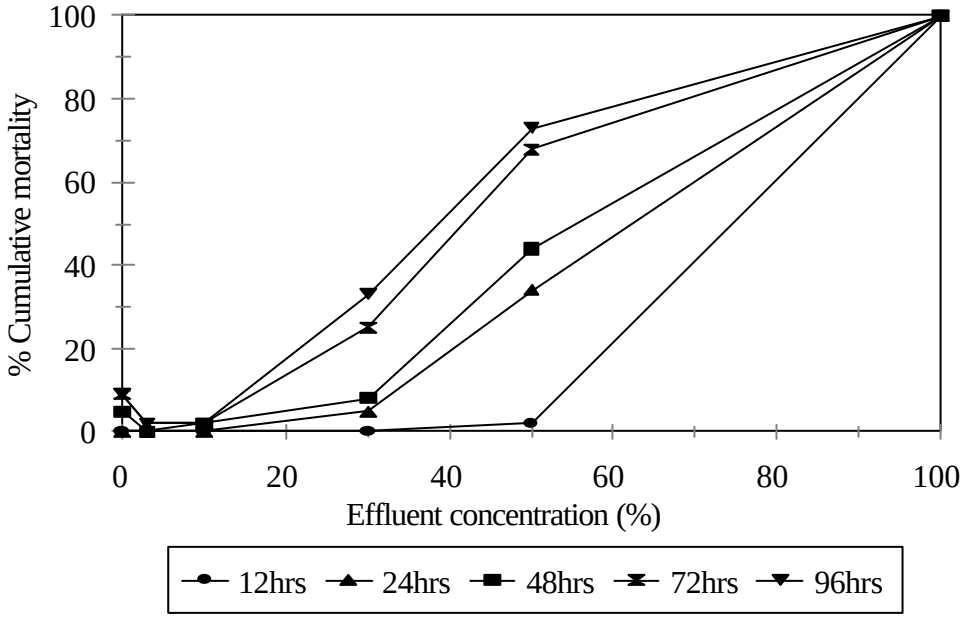

Figure 3.13 Experiment 5: Concentration-response curve for *T. tinctus* exposed to Genaral Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

73%, and for the 30% effluent concentration, mortality was less than 50%. Only 2% and 3% of organisms responded at 3% and 10% effluent concentrations respectively. The 1% effluent concentration was discarded due to very high mortality, which could not be explained. The concentration-response curve (Figure 3.13) showed that with the 100% effluent, all of the organisms died within 12 hrs, while with the 40% effluent concentration, half of the organisms died after 96 hrs. The 3% effluent concentration had no dead organism. The Probit LC50 was calculated at 38% effluent concentration. The TSK LC50 value was similar to the Probit LC50 value, with zero trim indicating good fit of the model (Table 3.4). This effluent was the second least toxic of the five GKE batches tested in this study.

Experiment 6 General Kraft Effluent (20-08-1998)

Description: *GKE Exp 6*

Responses of *T. tinctus* to the last GKE batch are shown in Figures 3.14 and 3.15. A full physico-chemical analysis of individual concentrations used in the experiment is presented in Appendix A, Table A4.

Water quality

Table 3.11 presents ranges of daily measured variables during Experiment 6.

TABLE 3.11
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 6

Para-Meter (mg/l)	% Effluent concentration						
	Control	**1**	**3**	**10**	**30**	**50**	**100**
EC (mS/m)	12.0-13.1	13.2-15.1	15.1-16.7	19.3-26.0	34.2-52.8	47.8-77.4	129.6-135.1
PH	7.3-7.8	7.4-7.9	7.4-7.9	7.4-7.9	7.5-7.9	7.3-7.9	6.8-7.9
Temp. (°C)	16.0-19.0	17.0-19.0	16.5-19.0	16.5-19.0	17.0-19.0	16.5-19.0	17.0-19.0

The temperature of the channels fluctuated between 16 and 19°C, varying by ± 2°C over the 96 hr test period. The pHs in the channels were within the accepted range (DWAF, 1991) and

did not change by more than one pH unit. This batch of effluent was characterized by an unusually high calcium concentration (203-206 mg/l). High levels of calcium reduce toxicity of trace metals such as copper (Dallas and Day, 1993). Sodium, sulphate and chloride levels were comparable to values in Experiment 5 (Appendix A, Table A2)). This effluent was less saline than the other effluents.

Toxicity results

In this experiment, there were no mortalities within the first 12 hours, except at 10% effluent concentration, which had 3% mortality. After 96 hrs, mortalities of the 100% and 50% effluent concentrations were 76% and 32% respectively. Generally, there was little response in lower effluent concentrations. Control mortality was zero. The Probit LC50 was calculated at 64% effluent concentration, and the TSK LC50 was 68% effluent concentration (Table 3.5). The responses in Experiments 5 and 6 were very different despite the fact that they were both GKE. The 100% effluent concentration response in Experiment 6 was almost the same as the 50% effluent concentration in Experiment 5. This could be attributed to high levels of calcium, which reduces toxicity. What was interesting was that the LC1 and LC5 values were similar.

In Experiments 7, 8, 9 and 10, *T. tinctus* were exposed to IKE over 96 hours.

Experiment 7 Irrigation Kraft Effluent (17-08-1998)

Description: *IKE Exp 7*

T. tinctus was exposed to IKE for 96 hrs at the full range (1-100%) of effluent concentrations. At the start of the experiment, the channel's mesh clogged due to high levels of suspended solids. Frequent brushing of the end mesh relieved the situation. Ranges of daily measured variables are shown in Table 3.12. The responses of *T. tinctus* are shown in Figures 3.16 and 3.17.

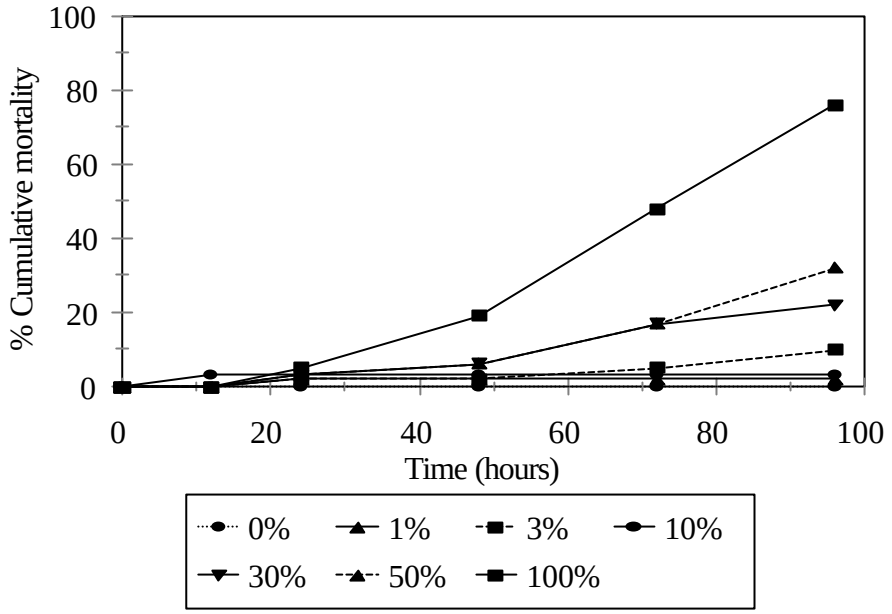

Figure 3.14 Experiment 6: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to General Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water.

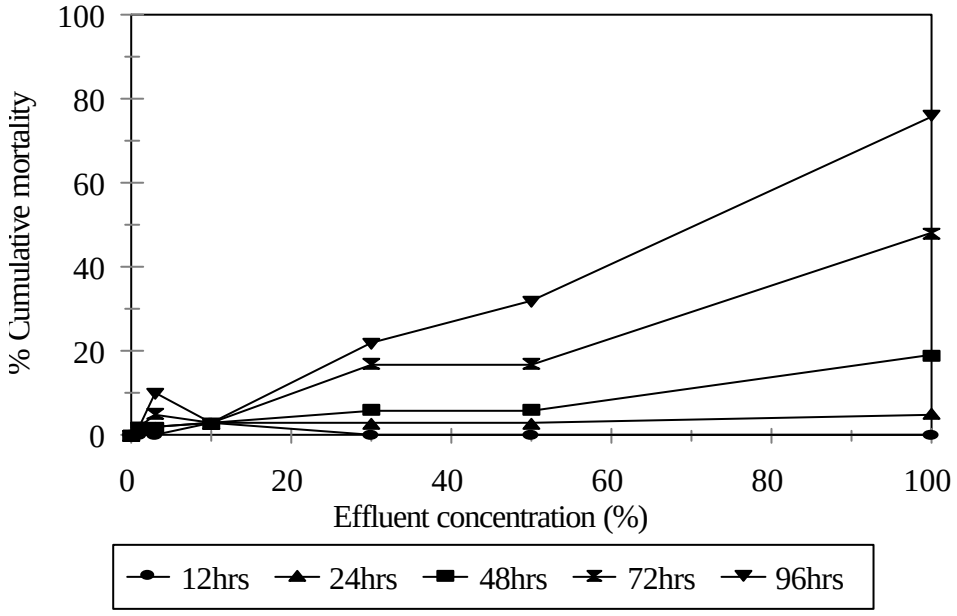

Figure 3.15 Experiment 6: Concentration-response curve for *T. tinctus* exposed to General Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

Water quality

Table 3.12 shows ranges of the daily measured variables during Experiment 7.

TABLE 3.12
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 7

Para-meter (mg/l)	% Effluent concentration							
	Control	**1**	**3**	**10**	**30**	**50**	**75**	**100**
EC (mS/m)	11.2-13.2	13.9-16.6	18.3-22.8	27.9-32.3	84.8-95.6	15.0-19.0	179.1-193.7	234.0-251.0
PH	7.5-7.8	7.7-7.7	7.5-7.7.8	7.5-7.8	7.1-7.7	6.5-7.6	5.3-7.5	5.1-5.8
Temp. (°C)	15.5-19.5	15.0-19.0	15.0-19.0	15.0-20.0	15.0-19.0	15.0-19 .0	15.0-18.0	15.0-18.0

The high concentrations of suspended solids contributed to increased turbidity. The pH in the channels showed a decreasing trend with increasing effluent concentrations. Temperatures within the channels fluctuated between 15.0 and 20.0°C, and varied by ± 5°C (Table 3.12). Variation in temperature could have affected the tolerances of the organisms. EC was high compared to the sampling site and the recommended limit for irrigation with industrial wastewater. Chloride concentrations were relatively higher than those of the general effluent experiments, possibly indicating the presence of organochlorines in the effluent. Sodium and sulphate concentrations were also high (Appendix A, Table A5), and may have contributed to salinity of this effluent. The nutrient concentrations were generally low. High values for Na, SO_4 and Cl concentrations showed that the effluent was saline.

Toxicity results

In Experiment 7, there was generally very little response within the first 12 hrs. Thereafter, 50% response occurred in the 75 and 100% effluent concentrations. By the end of 24 hrs, the 75% and 100% effluent concentrations had reached 100% mortality (Figure 3.16). At the end of 96 hrs, only the 75 and 100% effluent concentrations had reached 100% mortality.

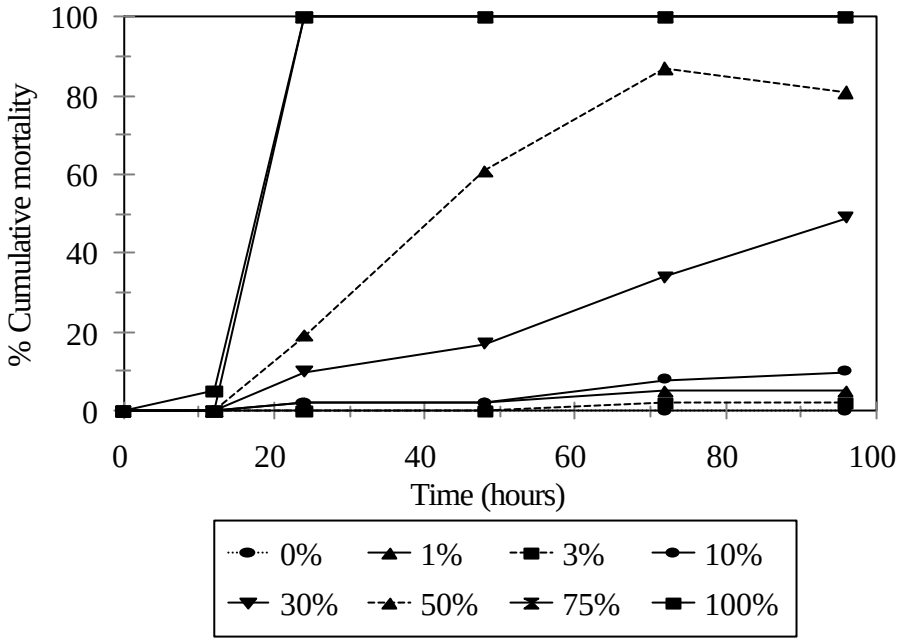

Figure 3.16 Experiment 7: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to Irrigated Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water.

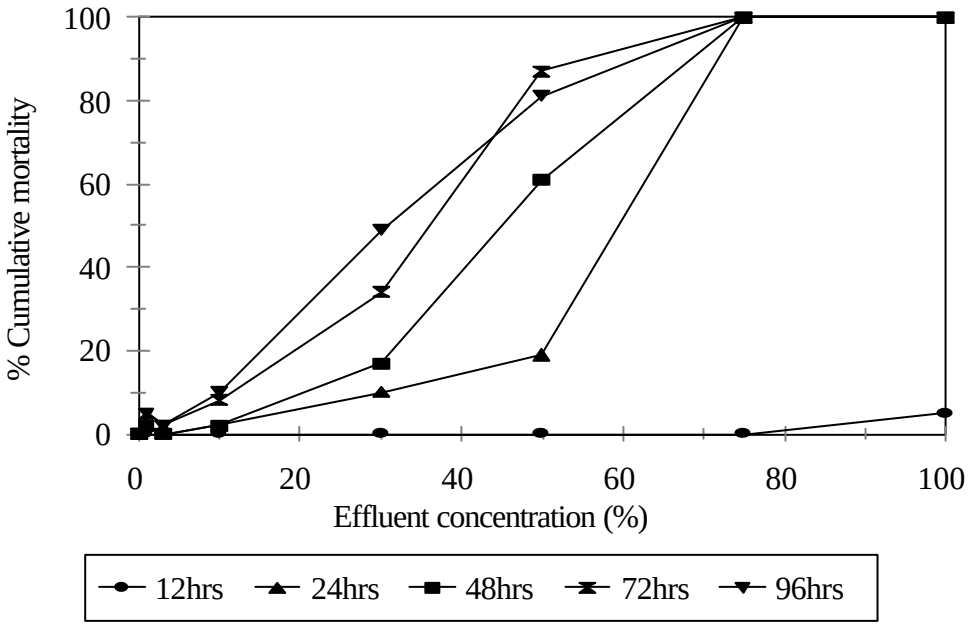

Figure 3.17 Experiment 7: Concentration-response curve for *T. tinctus* exposed to Irrigation Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

Effluent concentrations 1, 3 and 10 % responses were less than 10%. Control mortality was zero indicating the good quality of the river water. The concentration-response curve showed that at 75% effluent concentration all of the organisms had died after 48 hrs and that at 30**%** effluent concentration, half of the organisms had died after 96 hrs (Figure 3.17). The Probit LC50 was calculated at 32% effluent concentration with low chi-square heterogeneity, and the TSK LC50 was at 27% concentration (Table 3.5).

Experiment 8 Irrigation Kraft Effluent (17-08-1998)

Description: *IKE Exp 8*

T. tinctus were exposed to IKE for 96 hrs at the full range of effluent concentrations (1-100%). The channels became clogged despite having sieved the effluent before the start of the experiment. This was due to high levels of suspended solids in the effluent. Table 3.13 presents ranges of daily measured variables during Experiment 8. The responses of *T. tinctus* are showed in Figures 3.18 and 3.19. The full chemical analysis of individual concentrations is presented in Appendix A, Table A6.

Water quality

Table 3.13 presents ranges of measured variables during Experiment 8.

TABLE 3.13
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 8.

Para-meter (mg/l)	% Effluent concentration							
	Control	**1**	**3**	**10**	**30**	**50**	**75**	**100**
EC (mS/m)	11.2-13.2	14.1-16.1	24.1-27.9	31.1-35.6	84.6-97.2	129.4-148.3	182.6-194.3	236.1-247.3
pH	7.5-7.8	7.4-7.9	7.4-7.9	7.5-7.9	7.1-7.7	5.9-7.6	5.4-7.2	5.1-5.4
Temp. (°C)	15.5-19.5	15.0-19.0	14.5-18.0	15.0-19.0	15.0-18.0	14.5-18.0	15.0-18.0	15.5-18.5

The pH was acidic at high effluent concentrations. The temperature in the channels fluctuated between 14.5 and 19.5°C (Table 3.13), and varied by ± 4°C. EC of this effluent was well

above that of the sampling site (Table 3.3). This effluent was comparable to the batch used in Experiment 7.

Toxicity results

After 24 hrs, 100% mortality was reached in both the 75 and 100% effluent concentrations. The 50% effluent concentration also reached 100% mortality by the end of 72 hrs. Lower effluent concentrations (i.e. 1, 3 and 10%) were similar to control responses (Figure 3.18). This appeared to show that low effluent concentrations of this irrigation effluent do not elicit mortality responses. A concentration-response curve showed that at 75% concentration all of the organisms died after 24 hrs. At 50% effluent concentration, half of the organisms had died after 48 hours. At 3% effluent concentration no organisms had died (Figure 3.19). The Probit analysis could not calculate the LC50 and 95% confidence limits, as the response was not monotonic. The responses were very low at low effluent concentrations and suddenly shot up from 10 to 100% effluent concentrations.

Experiment 9 Irrigation Kraft Effluent (20-08-1998)

Description: *IKE Exp 9*

T. tinctus were exposed to seven concentrations of IKE for 96 hrs ranging between 1 and 100%. This batch of effluent also had high levels of suspended solids and the effluent was sieved successfully, so that clogging was not a problem during the experiment. Acclimation mortality was 2%. Only one set of readings was taken in the 100% effluent concentration, as 100% mortality was reached within the first 7 hrs of the experiment. The 10% effluent concentration was omitted in the LC50 calculations, as mortalities were completely anomalous. Figures 3.20 and 3.21 show the organisms responses during the experiment. A full analysis of individual effluent concentrations is presented in Appendix A, Table A7.

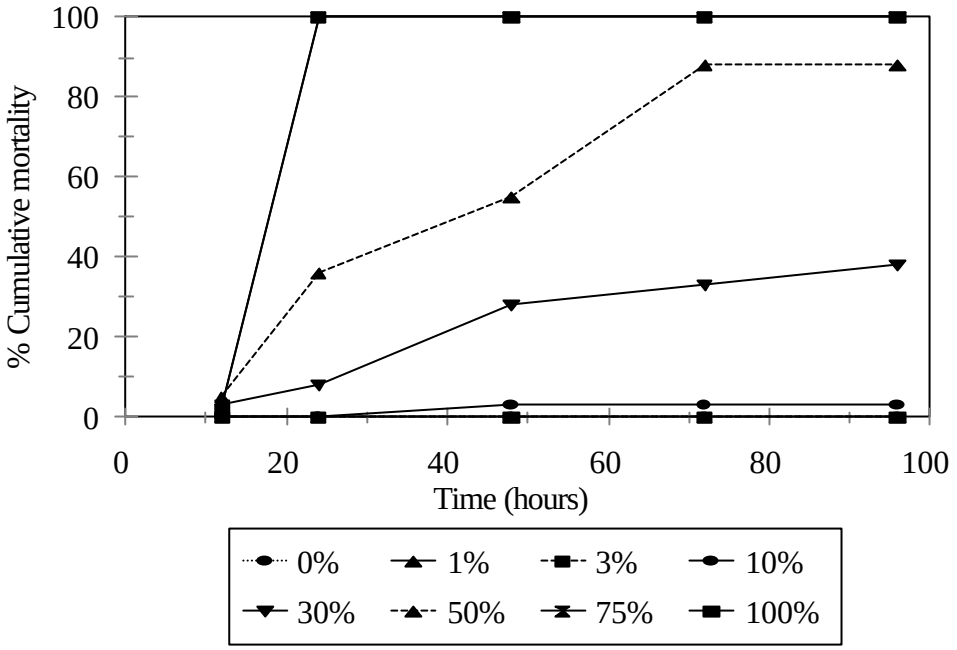

Figure 3.18 Experiment 8: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to Irrigation Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water.

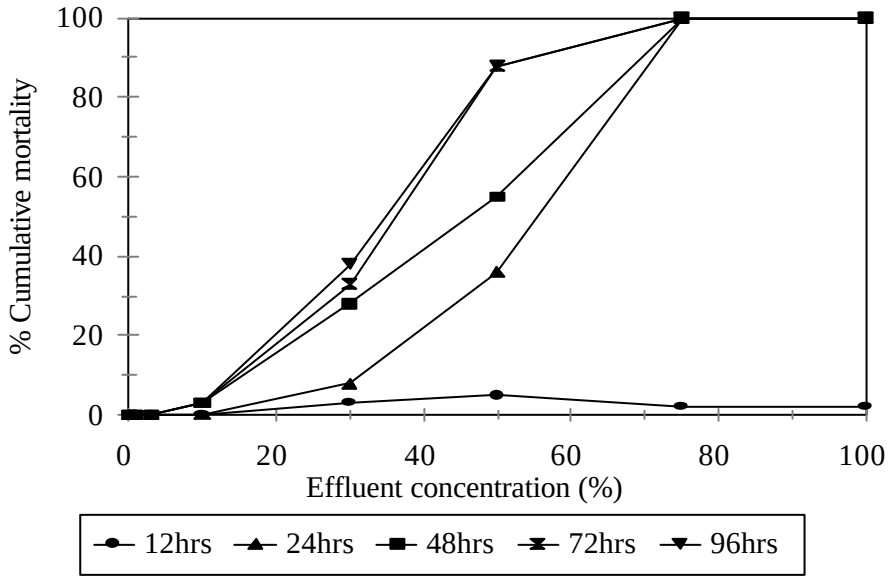

Figure 3.19 Experiment 8: Concentration-response curve for *T. tinctus* exposed to Irrigation Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

Water quality

Table 3.14 presents ranges of daily measured variables during Experiment 9. Only one reading was taken in the 100% effluent concentration, as it had reached 100% mortality within the first 7 hrs.

TABLE 3.14
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 9. * INDICATES THAT ONLY ONE READING WAS TAKEN.

Para-meter (mg/l)	% Effluent concentration							
	Control	**1**	**3**	**10**	**30**	**50**	**75**	**100**
EC (mS/m)	12.0-13.2	16.3-17.7	23.5-25.6	46.7-51.9	114.1-124.8	177.4-193.7	249.8-255.3	320.3*
PH	7.3-7.8	7.4-7.8	7.4-7.8	7.4-7.8	7.3-7.9	7.0-7.8	6.5-7.1	6.0*
Temp. (°C)	16.5-18.5	17.0-19.0	16.5-18.0	16.5-18.5	17.0-19.0	17.5-19.0	17.5-18.0	18.5*

The temperatures within each channel fluctuated between 16.5 and 19.0°C over the 96 hrs toxicity test period, varying by about ±2°C within each channel. The pH in the channels was around neutral (Table 3.14). EC was higher than the GKE, indicating that the effluent was one of the most saline of the effluents tested in this study.

Toxicity results

In this experiment, the 100% effluent concentration reached 100% mortality within the first 5 hours of the start of the experiment. The 50% effluent concentration reached 100% mortality after 96 hrs. The 10% effluent concentration was discarded, as it presented abnormally high mortalities after day 3. A concentration-response curve showed that at 48% effluent concentration, all of the organisms had died after 96 hrs. At 30% effluent concentration half of the organisms had died after 96 hours (Figure 3.21). The LC50 could not be calculated by Probit analysis. This seemed to have been influenced by the number of concentrations used to calculate the LC50. However, the TSK analysis showed the LC50 at 19% effluent concentration, which overlaps with the GKEs (Table 3.5).

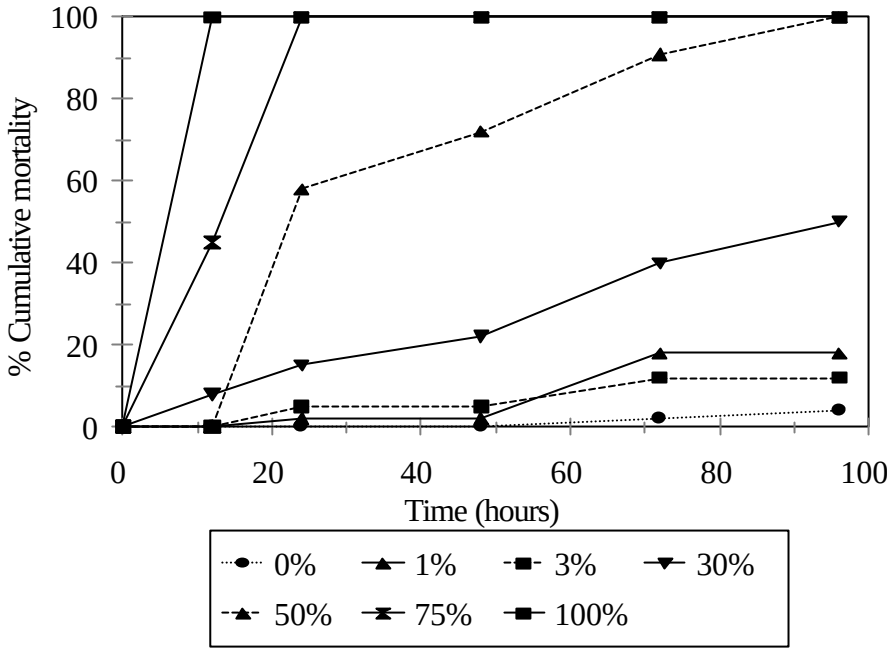

Figure 3.20 Experiment 9: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to Irrigation Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water.

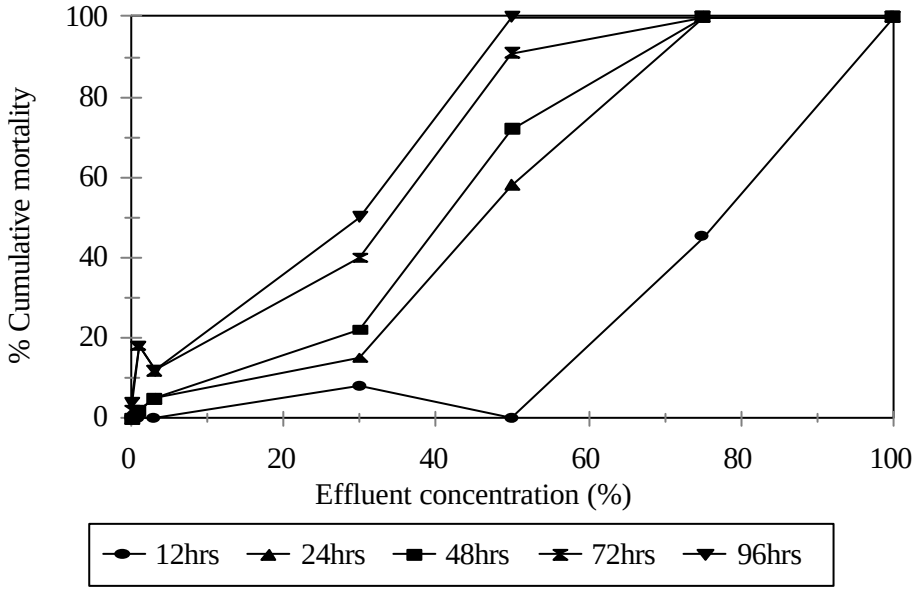

Figure 3.21 Experiment 9: Concentration-response curve for *T. tinctus* exposed to Irrigation Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

Experiment 10 Irrigation Kraft Effluent (20-08-1998)

Description: *IKE Exp 10*

T. tinctus were exposed a range of IKE concentrations for 96 hrs. Mortality after acclimation was 4%. Responses of *T. tinctus* are showed in Figures 3.22 and 3.23. A full chemical analysis for individual effluent concentrations is shown in Appendix A, Table A8.

Water quality

Ranges of daily measured variables during Experiment 10 are shown in Table 3.15.

TABLE 3.15
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 10.

Para-meter (mg/l)	% Effluent concentration							
	Control	1	3	10	30	50	75	100
EC (mS/m)	12.0-13.1	15.9-17.6	23.5-26.2	45.7-50.6	113.6-125.3	178.8-199.5	250.4-255.6	313.7-325.4
pH	7.3-7.8	7.3-7.8	7.4-7.8	7.4-7.8	7.3-7.8	7.0-7.8	6.6-7.4	6.3-7.4
Temp. (°C)	16.5-18.5	16.5-18.0	16.5-18.0	16.5-18.0	16.5-18.0	16.5-18.0	17.0-18.0	18.0-18.5

The channel temperatures fluctuated within ± 2°C ranging between 16.5 and 18.5°C. The pH was within the General Standards (DWAF, 1991) and similar to the batch of effluent used in Experiment 9 (Table 3.15), although EC was higher than the GKE. The chloride, sodium and sulphate levels were much higher than the GKE (Appendix A, Table A8), and contributed to the salinity of the effluent.

Toxicity results

Control mortality was 2%. The 75 and 100% effluent concentrations reached 100% mortality after 24 hrs (Figure 3.22). Effluent concentrations 1, 3 and 10%, had a minimal effect on the test organisms. A concentration-response curve showed that at 75% effluent concentration, all of the organisms had died within 24 hrs; and at 30% effluent concentration, half of the

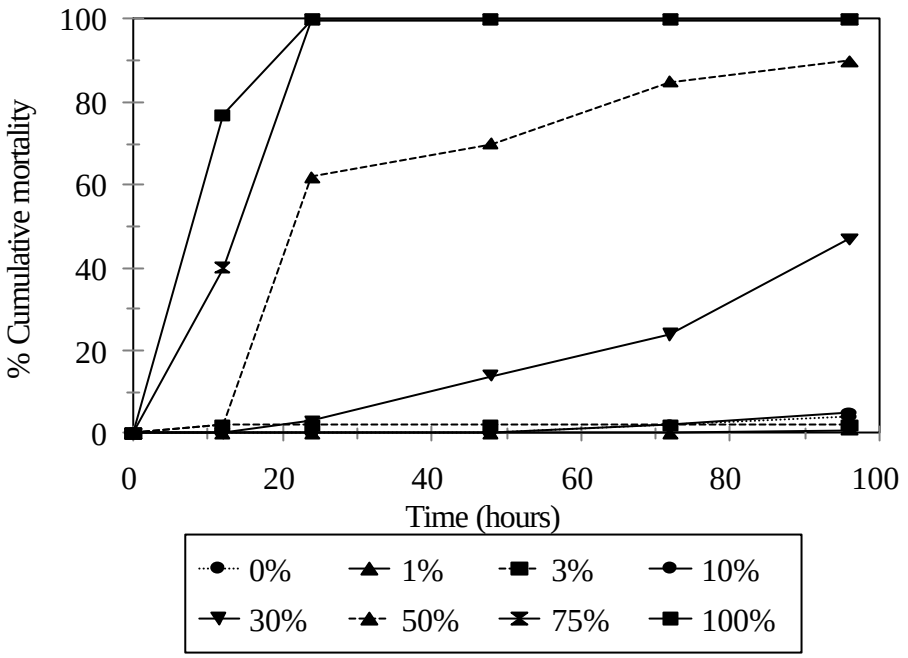

Figure 3.22 Experiment 10: The percentage cumulative mortality of *T. tinctus* over 96 hrs, after exposure to Irrigation Kraft Effluent at a range of effluent concentrations. The diluent was Sabie River water.

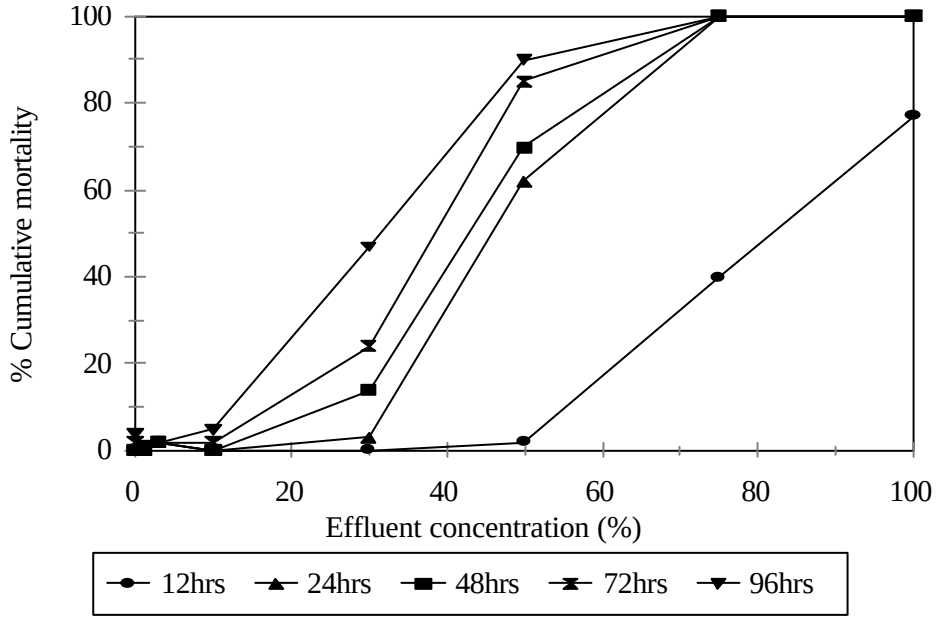

Figure 3.23 Experiment 10: Concentration-response curve for *T. tinctus* exposed to Irrigation Kraft Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Sabie River water.

organisms had died after 96 hrs (Figure 3.23). The Probit LC50 was calculated at 32% effluent concentration and was similar to the batch used in Experiment 7, which is also an irrigation effluent (Table 3.5). The TSK analysis also indicated a similar response.

General comments on 1998 experiments

Effluent batches for Experiments 5 and 6 were different from each other, chemically and in responses, although they were both GKE. This showed variability in the effluent. This should be expected since the effluent does not go through a retention period for stabilization. All four IKE responses were similar, even though experiments were conducted at different times. Chloride levels were the highest in the IKE.

3.5.4 Analysis of water quality data

Water samples were taken at the start and the end of each experiment and analyzed by IWQS. The physico-chemical results are summarized in the form of ranges in Table 3.4. The chemical profile of each effluent batch used for each experiment are presented in Appendix A.

Sample analysis showed variation in most parameters during the experiments for both GKE and IKE. The IKE had higher TDS levels than the GKE. EC for both GKE and IKE was higher than the recommended limit of 200 mS/m, for water used for irrigation (DWAF, 1999), and ranged from 310 to 395 mS/m, and 101 to 425 mS/m respectively. These values are almost 20 times higher than those of the sampling site. Turbidity and suspended solids were high and could be possible contributory factors to mortality. The pH was within General Standards range (6.5-9.5) (DWAF, 1991) most of the time, but variation was above the recommended 1.0 pH unit (Dallas and Day, 1993). Nutrient levels showed an increasing trend as percentage effluent concentration increased, and also remained within the General Standards limits (DWAF, 1991). Experiments 7, 8, 9 and 10, showed that chloride, sodium and sulphate levels were ten times higher than those of the sampling site. Trace metals remained insignificant in all the experiments. The whole effluent was analysed at the beginning and at the end of the experiment, and the results showed slight differences in variables, which indicated no measurable degradation of the effluent over time.

3.5.5 Site-specific whole effluent guidelines for kraft effluent

As mentioned in Chapter 2, Environmental Risk Assessment (ERA) is a tool that can assist during environmental decision-making. The focus of environmental protection is at the level of the resource at risk. The US EPA Guidelines (US EPA, 1998) describe single-species chemical-based risk assessment techniques for assessing risks to ecosystems from multiple stressors and multiple endpoints (Murray and Claassen, 1999). The DWAF, in collaboration with the CSIR, is in the process of developing an ERA framework for South Africa (Murray and Claassen, 1999). The data from this study can contribute to describe a single-species, hazard-based assessment of the effluents studied.

Palmer and Scherman (in press) 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 effluent. For each experiment, the tolerance end-points described were LC1, LC5, and LC50 values. The lower and the upper 95% confidence limits of the LC1, LC5 and LC50 values are listed (Tables 3.16 and 3.17), except for Experiment 8 and 9, for which the Probit analysis method was not appropriate and results not available.

Tables 3.16 and 3.17 therefore present LC1, LC5, LC50 values and their 95% confidence limits, plus the calculated AEV for each GKE and IKE experiments respectively.

In this study, chronic tolerance tests were not undertaken. The ranked hazard assessment tables start with Class A (minimal risk) at effluent concentrations below any result derived from acute experimental results. The tolerance end-points for each experiment were then ranked and associated with a particular predicted in-stream river health class (Appendix C).

Table 3.18 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. Ranked lists for Experiments 2-10 (Tables C1-7), are presented in Appendix C.

TABLE 3.16
LC1, LC5 AND LC50 VALUES OF THE PROBIT ANALYSIS FOR THE INDIVIDUAL GENERAL KRAFT 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	LC50 95% 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

TABLE 3.17
LC1, LC5 AND LC50 VALUES OF THE PROBIT ANALYSIS FOR THE INDIVIDUAL IRRIGATION KRAFT EFFLUENT EXPERIMENTS, THEIR 95% CONFIDENCE LIMITS AND AEV. (UCL = UPPER CONFIDENCE LIMITS, LCL = LOWER CONFIDENCE LIMITS).

Acute (96hr) IKE	LC1	LC1 95% LCL	LC1 95% UCL	LC5	LC5 95% LCL	LC5 95% UCL	LC50	LC50 95% LCL	LC50 95%UCL	AEV
Experiment 7	12.2	6.3	17	16.2	9.7	26.2	31.9	26.2	36.3	6.1
Experiment 8	LC1, LC5 AND LC50 VALUES NOT AVAILABLE									
Experiment 9	LC1, LC5 AND LC50 VALUES NOT AVAILABLE									
Experiment 10	14.2	7.7	18.9	17.9	11.2	22.5	31.5	26.5	35.3	7.1

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.

TABLE 3.18

EXPERIMENT 1, GKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2) AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1) AND HAZARD-BASED DESCRIPTIONS (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN (IN PRESS).

Tolerance test end-points	% effluent concentra-tion	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 LC1 lower 95% CL LC1	1.9 2.3 3.8	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
LC5 lower 95% CL LC1 upper 95% CL	4.2 5.0	Moderate hazard to intolerant biota: 95% probability of mortality between 1-5 % after acute exposure	C	4.0 – 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

CL: confidence limit

Table 3.19 gives a summary of suggested guidelines for the kraft effluents tested. As shown by Table 3.19, 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.

TABLE 3.19

INDIVIDUAL KRAFT EFFLUENT EXPERIMENTS WITH ASSOCIATED RIVER HEALTH CLASSES AND ASSIGNED PERCENTAGE EFFLUENT CONCENTRATION GUIDELINE RANGES.

River healthc lass	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 10
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

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. This table shows the relative similarity of the toxicity, and the required concentration of the 1997 GKE and 1998 GKE and IKE samples. The 1998 GKE and IKE samples showed lower toxicity than the 1997 GKE samples. This seemed to relate to effluent chemistry (Appendix A).

At present, IKE is used at 100% concentration. Table 3.19 indicates that to reduce potential environmental risks, effluent should be diluted. These in-stream percentage effluent concentrations could be used to calculate the volume of effluent which should reach the groundwater and river.

3.5.6 Groundwater

Experiments 11 and 12 Groundwater (29-07-1998)

Description: *Groundwater Exps 11 and 12*

These experiments were conducted as a short-term chronic toxicity tests over 12 days, using groundwater as the toxicant and the same test organisms. *T. tinctus* were exposed to groundwater at a range of mixtures, with Sabie River water as the diluent and the control; and also to 100% groundwater. Two channels (duplicates) were run per concentration, analyzed separately as Experiments 11 and 12 (Table 3.5). Responses of *T. tinctus* are shown in Table 3.20 and Figure 3.24. The results of the two experiments are given as means. Control mortality was 6% after 12 days.

Table 3.20 shows groundwater percentage response and the means of duplicate channels at 4, 7, 10 and 12 days. Figure 3.24 shows the mean cumulative mortality of *T. tinctus* exposed to groundwater over 12 days. Responses showed that groundwater caused some toxicity at high concentrations.

TABLE 3.20
GROUNDWATER PERCENTAGE MORTALITY AT 4, 7, 10 AND 12 DAYS. THE RESULTS OF DUPLICATE CHANNELS, AND THE MEAN, ARE GIVEN.

Ground-water (%)	4 Days			7 Days			10 Days			12 Days		
	Duplicates		Mean	Duplicates		Mean	Duplicates		Mean	Duplicates		Mean
0	2	4	**3**	4	4	**4**	6	6	**6**	6	6	**6**
10	0	7	**3.5**	3	7	**5**	5	20	**12.5**	5	20	**12.5**
25	13	2	**7.5**	13	2	**7.5**	13	2	**7.5**	13	2	**7.5**
50	5	5	**5**	33	8	**21**	33	16	**24.5**	42	19	**30.5**
100	5	39	**22**	16	45	**31**	21	55	**38**	23	60	**40.5**

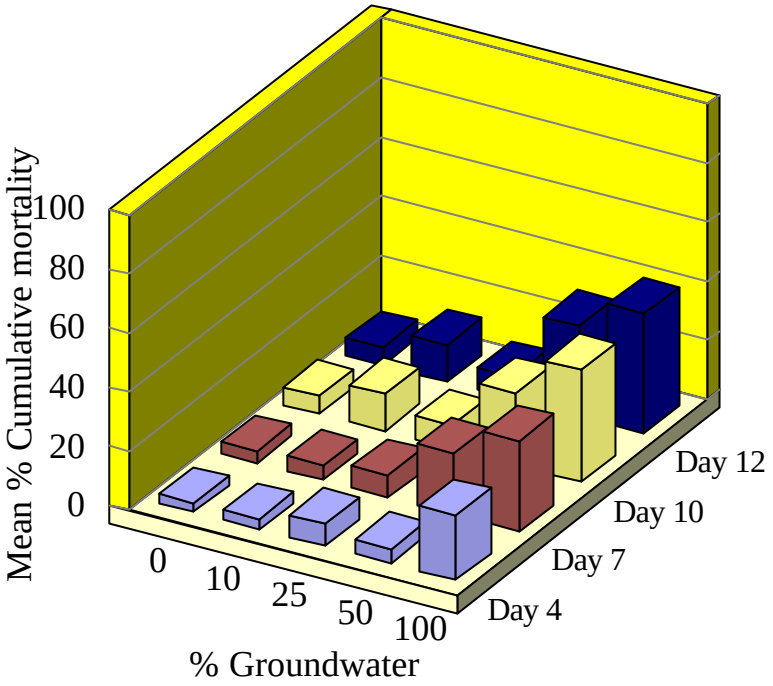

Figure 3.24 Mean cumulative mortality of *T. tinctus* over 12 days, after exposure to groundwater at 10, 25, 50 and 100% concentrations. The diluent was Sabie River water.

Water quality

Tables 3.21 and 3.22 show the ranges and means of daily measured variables for each mixture of Experiment 11 and 12 respectively, during 12 days groundwater toxicity testing. Samples were taken at 0 hrs, 96 hrs, day 8 and day 12, for chemical analyses (Appendix D).

The pH within the channels ranged from 7.5-8.3 with a variation of ± 0.3 pH units in each channel. The means showed pH increasing with increase in groundwater mixtures (Table 3.21 and 3.22). The mean temperatures ranged between 16.5 and 17.5°C and showed consistency, however temperatures within individual concentrations fluctuated widely over 96 hours. EC was about 10 times that of the receiving water (Table 3.3) and was higher than the General standard of 75 mS/m (DWAF, 1991). Chloride levels in groundwater fluctuated between 227 and 264 mg/l, which were about the same as the effluent concentrations, and may show the impact of the IKE (Appendix D, Tables D1, D2). Nutrient levels were insignificant. Trace metals have not been shown because they were below detectable limits.

TABLE 3.21
RANGES OF DAILY MEASUREMENTS OF GROUNDWATER PER CHANNEL (EXPRESSED AS % CONCENTRATION) WITH THEIR MEANS DURING A CHRONIC (12 DAY) GROUNDWATER TOXICITY TESTING FOR EXPERIMENT 11. A RANGE AND A MEAN FOR EACH GROUNDWATER MIXTURE ARE GIVEN

Parameter (mg/l)	Control		10		25		50		100	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
EC (mS/m)	10.8-13.7	**11.3**	23.4-27.3	**24.8**	40.1-45.7	**42.3**	66.5-78.3	**71.9**	111.8-121.6	**112**
pH	7.5-7.8	**7.6**	7.3-8.1	**7.8**	7.8-8.2	**7.4**	8.1-8.3	**8.2**	8.0-8.3	**8.2**
Temp. (°C)	14.0-20.0	**17.7**	15.0-19.8	**17.5**	15.0-19.5	**16.5**	14.0-19.0	**17.2**	14.0-19.0	**17.4**

TABLE 3.22
RANGES OF DAILY MEASUREMENTS OF GROUNDWATER PER CHANNEL (EXPRESSED AS % CONCENTRATION) WITH THEIR MEANS, DURING A CHRONIC (12 DAY) GROUNDWATER TOXICITY TESTING FOR EXPERIMENT 12. A RANGE AND A MEAN FOR EACH GROUNDWATER MIXTURE ARE GIVEN.

Parameter (mg/l)	Control		10		25		50		100	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
EC (mS/m)	10.8-13.7	**11.3**	24.0-27.0	**24.8**	41.0-47.5	**43.3**	67.2-76.7	**71.3**	108.9-123.0	**116**
pH	7.5-7.8	**7.6**	7.8-8.1	**7.9**	7.9-8.2	**8.0**	8.0-8.3	**8.2**	8.1-8.3	**8.2**
Temp. (°C)	14.0-20.0	**17.7**	13.8-19.5	**17.2**	14.0-19.0	**17.1**	14.0-19.5	**17.2**	14.0-19.5	**17.0**

3.5.7 The main findings of this study

The mayfly nymph *T. tinctus* from the Sabie River in the sub-tropical low-veld region of South Africa showed sensitivity to both general and irrigation kraft effluents. Groundwater has also showed some lethal effects to aquatic biota at high 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 (Tables 3.5, 3.16, 3.17). Interestingly, GKE samples from 1998 were less toxic than those tested in winter 1997 (Table 3.5). Generally, toxicity of both kraft effluents was high compared to groundwater (Table 3.5). The IKEs were more saline than groundwater, which was more saline than the Elands and the Sabie Rivers. Groundwater toxicity was measurable (Table 3.20), and therefore groundwater contamination was evident.

3.6 DISCUSSION

3.6.1 Introduction

The aim is to discuss the results of this study in the context of national and international literature and the philosophy of South African water quality management. The main aim of the study was to investigate the potential effects of pulp and paper kraft mill effluent on indigenous riverine mayfly nymphs, and to report on the relative toxicity of General (GKE) and Irrigation (IKE) Kraft Effluents and the recipient groundwater. To achieve this aim, mayfly nymphs (*T. tinctus*) were exposed for 96 hours to a range of kraft effluent concentrations, and for 12 days to a range of groundwater mixtures. The secondary aim was to provide a set of hazard assessment guidelines, which relate effluent toxicity to river health class.

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.

The results of this study were used in the application of a derived hazard assessment guideline, which provides a recommended IKE dilution factor (Table 3.19), which would render the IKE acceptable in-stream, at different levels of ecosystem health. This information could be used in the decision-making about IKE treatment and irrigation.

In the introduction, the question of the effects of pulp and paper kraft effluent on aquatic environments was raised in the form of three questions: Why are pulp and paper effluents toxic? What are the environmental and biological effects? What end-points have been used to detect these effects? It would be useful to consider these questions in the light of this study.

3.6.2 Effects of pulp and paper kraft effluent on aquatic invertebrates

Why are pulp and paper effluents toxic?

The chemical source of kraft effluent toxicity has been much debated and this is discussed at length in Section 3.1.3.

Bleaching

Despite the fact that the kraft mill investigated during this study mainly used ClO_2 and ozone for bleaching, the effluent was still acutely toxic. Chlorine-free bleaching results in very little formation of polychlorinated compounds. The biological treatment further reduces the toxic potential of the kraft effluent (Haley *et al.*, 1995; Oikari and Holmbom, 1996). Since effluents used for the study undergo primary treatment and pH stabilization before being irrigated, but not secondary treatment, organochlorines could still be contributing to effluent toxicity (Swanson, 1996). A complete substitution of chlorine dioxide has resulted in reduced impacts on aquatic ecosystems (Gullichsen, 1991; Haley *et al*., 1995; Landner *et al*., 1994; Soimasuo *et al*., 1998), by reducing chlorinated phenolic and dioxin/furan formation to levels at or below the detection limits (Swanson *et al*., 1996; Oikari and Holmbom, 1996), and altering the chemical composition of the effluent (Gullichsen, 1991; Servos *et al*., 1996).

Generally, bleaching effluent is acidic due to the use of strong acids and the reaction end-products during the bleaching process, and these are the major source of toxicity in kraft effluents. The acidic bleaching effluent was mixed with GKE to form IKE, and IKE is treated with $CaCO_3$ before it is irrigated, hence the pH of IKE of this study was towards neutral. If bleaching effluents were to enter the aquatic environment, and alter the pH, this could affect the rate and type of ion exchange across the gills of organisms (Dallas and Day, 1993). Even effluent directly from the pipeline will definitely have an impact on aquatic biota should it reach the aquatic environment. Therefore, it is very important that strict precautionary measures be taken to avoid any leaks from the pipeline or overflows from the clarifying tanks.

Comparison of GKE and IKE

Toxicity 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 (Tables 3.5; 3.16; 3.17). Therefore, IKE was as potentially toxic to aquatic biota as the GKE during 1998. This supports the information stating that there is little difference in toxicity of bleached versus unbleached mill effluents to aquatic life (Eysenbach *et al*., 1990; Smith and Sprague, 1992; Robinson *et al*., 1994; Eklund *et al*., 1996). This could show that toxicity has little to do with chlorination, as effluent without chlorine was also toxic. Effluent chemistry did not differ by a wide margin, except for sodium, chloride and sulphate levels in IKE, which were much higher than GKE.

Salinisation

Chemical analysis of surfaced groundwater indicated salinisation impacts. Exposure of test organisms to groundwater in a short-term chronic test (12 day) showed a demonstrable level of mortality. Salinisation was almost certainly linked to these lethal effects. Conductivity and TDS levels of both GKE and IKE were higher than the reference site, indicating salinity of the effluent could have contributed to test organisms' mortality. Electrical conductivity has been found to be a major contributor to *T. tinctus* mortality, with sulphate having a synergestic and calcium an antagonistic effect on mortality (Scherman *et al*. (in press)). Sulphate levels were above the guidelines for the protection of aquatic ecosystems (DWAF, 1996f). The results of this study indicate that salinity is a major contributor to the toxicity of kraft effluent.

In this study, as in that of Robinson *et al*. (1994), effluent toxicity was mostly related to the degree of effluent concentrations, i.e. as the effluent concentrations increased, more organisms died. At higher effluent concentrations, all responses were acute (within 24 hrs), except for Experiments 2 and 6. This showed that if the effluent entered the river during low flow, where the dilution factor will be low, devastating impacts could occur, as happened during an accidental spill in 1989 (Kleynhans *et al*., 1992). At lower effluent concentrations, toxicity was reduced, indicating that the effluent must be very dilute, in order to safely enter the aquatic environment.

Treatment

In this study, pulp and paper kraft mill effluents were generally found to be acutely toxic. This could be attributed to the fact that there is no secondary treatment or any form of biological treatment of effluents in the mill, before effluent is released for irrigation. Studies have shown that untreated or inadequately treated effluents from any pulping process have the potential for significant adverse environmental impact (Ahtianen *et al*., 1996; Smith and Sprague, 1992; Eklund *et al*., 1996). Biological treatment effectively reduces acute toxicity of the effluent (Eysenbach *et al*., 1990; Kovacs *et al*., 1995; Priha, 1996). This is supported by Zanella and Berben (1980), Hodson *et al*. (1992) and Ahtianen *et al*. (1996), who found untreated bleached effluents were toxic to fish, but that biological treatment reduced acute toxicity. The type of wood used or natural constituents of wood also influences the toxicity of the effluent produced (Ahtianen *et al*., 1996; Axeg@rd *et al*., 1993; Verta *et al*., 1996).

What are the environmental and biological effects of discharging kraft effluents and what end-points can be used to detect these effects?

This is not a study on environmental effects, but the exploratory investigation of groundwater indicated the potential hazards of irrigating kraft effluent. Table 3.20 and Figure 3.24 showed clearly that organisms responded negatively to the higher concentrations of groundwater. It would usually be difficult to undertake such a study because the chemistry of the groundwater is so different from surface water. However, in this case the groundwater had surfaced naturally and was collected from a surfaced spring.

The main chemical difference between groundwater and Sabie River water is salinity, particularly the sodium, sulphate and chloride levels. Although groundwater had a salinity range of 109-143 mS/m versus 13.0-15.8 mS/m in the receiving water, the implication that groundwater has been impacted by irrigation with kraft effluent is correlative. However, exposing *T. tinctus* to elevated salinities, Palmer and Scherman (in press) suggested that salinities above 50 mS/m would result in Class E/F conditions in the Sabie River, and thus constitute an unacceptable risk to sensitive biota. The Elands and Sabie Rivers share a similar natural salinity profile (Table 3.3), therefore it is likely that the salinisation of the groundwater to

109-143 mS/m could pose an environmental threat. The difficulty of groundwater remediation is an exacerbating factor.

A consideration of biological effects on the basis of acute toxicity results is difficult, but this study clearly showed kraft effluents have the potential to impact negatively on biota. This was demonstrated by the 1989 spill (James and Barber, 1991; Kleynhans *et al*., 1992), where large populations of biota were destroyed, but have since recolonised. The mill is now carefully and protectively managing the effluent, and irrigation is the only routine route of exposure. Given that in this study only one species was exposed to kraft effluent over an acute time period, and that no study was undertaken of chronic or community responses, it is necessary to determine what can be concluded about the potential biological effect of irrigation kraft effluent.

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.

Since acute toxicity testing is relatively cost effective (Cairns, 1983; Rand, 1995), it is advantageous to infer chronic and sub-lethal effects from acute lethal data. By taking the LC1, LC5 and AEV as the basis of a hazard assessment guideline for kraft effluent disposal, we are attempting to extrapolate acute effects to the likely biological effect on the environment. We have therefore linked low levels of response to changes in feeding and breeding, as it seems reasonable to infer the possibility of these effects from actual mortality data.

3.6.3 Effluent and organism variability

Effluent toxicity could be attributed to several factors. Firstly, the production of pulp involves a multitude of factors such as wood species, type of pulping process and its efficiency. These determine the composition of the effluents produced. The effluents used in the study showed some variability, indicating a possibility of wood species being a contribution factor, since the pulping process remains the same. Secondly, the type of effluent treatment also influences the

chemical mixture of the effluent to be discharged (Lehtinen, 1996). These factors result in pulp and paper kraft effluents containing a complex of compounds that have different effects on aquatic environment (McLeay, 1987; Lowell *et al*., 1995). Thirdly, the effluent was discharged from a holding dam with little retention time before use for irrigation. A long retention period helps to stabilize the effluent, thus reducing variability (Grothe *et al*., 1996).

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, in press). Toxicity tests were conducted using field-collected organisms, which constitute a wild population. The test population of *T. tinctus* has been subjected to reference toxicants (NaCl and Na_2SO_4) for several years where the acute and sub-chronic toxicity was measured (Palmer and Scherman, in press). LC50 values for Na_2SO_4, in terms of EC, ranged from 186-358 mS/m (mean 291 mS/m). LC1 and LC5 values from the same experiments showed less variability (LC1 values for Na_2SO_4 ranged from 22.3-24.5 mS/m; LC5 values ranged from 41.5-53.7 mS/m) (Palmer and Scherman, in press). As LC1 and LC5 values are more conservative, the use of these values may reduce the variability associated with the use of a wild population.

3.6.4 Application of WET testing in South African water quality management

Given that the use of a wild population of invertebrates as toxicity test organisms aims to provide greater site-specific relevance, it would be useful to consider how the results of this study can be used in South African water quality management.

Since 1994, South African water resource management has been comprehensively reviewed, culminating in the National Water Policy (DWAF, 1997a), and the National Water Act (No. 36 of 1998). One of the main principles on which the National Water Act is based is that of resource protection to ensure *sustainable* resource use. Resource protection is effected by the dual application of *resource-directed measures*, such as quantification of the ecological Reserve and *source-directed controls*, such as defining the conditions for licences. In both these approaches, the SAWQG (DWAF, 1996f), and General and Special Standards (DWAF, 1991), are well placed to provide information for the management of single substances.

Acute and chronic toxicity testing of single toxicants is well established (Rand, 1995), and is the basis of SAWQG for the protection of aquatic ecosystems (DWAF, 1996f). Guidelines for the protection of aquatic ecosystems detail the procedure for the use of acute and chronic toxicity test results in the derivation of water quality management criteria (DWAF, 1996f; Roux *et al*., 1996). These numerical values can be used to guide and assist in the formulation of effluent discharge licenses, which, according to the National Water Act (No 36 of 1998), will be strictly enforced. The use of national criteria for the aquatic environment, which are expressed as Acute Effect Value (AEV) and Chronic Effect Value (CEV), aims to provide adequate protection with only a small possibility of over-protection (Roux *et al*., 1996). This however requires information on the tolerances of aquatic biota (Palmer and Scherman, in press).

However, the government gazette (No 20526 of 1999), excludes complex effluent from the General Authorization (DWAF, 1999b), thus laying the foundation for a toxicity-based approach for the management of complex effluents. This is based on the recognition that complex mixtures have integrated effects on biota, compared with the effects of their individual constituents (Grothe *et al*., 1996).

The decision by DWAF to include WET testing into its toxic effluent management policy (Palmer and Jooste, conf. draft) has necessitated suitable procedures to be established for use in the South African situation (Slabbert *et al*., 1998a; DWAF, 2000). Slabbert *et al*. (1998a) have developed methods for WET testing for use in South Africa. Both ecological field observations, such as biomonitoring, and toxicological studies, should be used to provide a more accurate assessment of the impact of pollution on riverine organisms (Palmer *et al*., 1996). According to Kovacs and Megraw (1996), a toxicological approach is a cost-effective way of assessing effluent toxicity; acute toxicity testing being the most cost-effective.

This study aimed to investigate the application of WET testing of complex kraft effluents using indigenous test organisms to assess the potential effects of these effluents on riverine ecosystems. The WET would then be used in testing results in the development of hazard-based guidelines, for the disposal of kraft effluent into the environment. The study represents a first step in developing WET testing using indigenous riverine organisms. WET testing using indigenous riverine organisms can play an important role in auditing licenses. Therefore, it is fundamentally important to have some knowledge of the relationship between the results of laboratory toxicity tests and the actual responses in the receiving water.

Since both effluents were found to be acutely toxic, this study suggests that the kraft mill should focus on Environmental Risk Assessment (ERA) 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 the 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. An increase in turbidity and SS reduces light penetration, decreases primary production and food availability to organisms (Dallas and Day1993).

To conclude, the results of the study can be of use to the management of the Mpumalanga kraft mill. There is a clear indication that both GKE and IKE are variable and acutely toxic. Although the mill is using partly ozone and partly chlorine dioxide for bleaching, irrigation effluent is still toxic. This gives an indication that organochlorines are not the only possible contributors of toxicity. The fact that there is no secondary biological treatment before the effluent is used for irrigation could be contributing to the toxicity. The studies reviewed in this Chapter have indicated that secondarily treated effluent is less toxic and even non-toxic to aquatic biota. Groundwater has been shown to have some lethal effects to aquatic biota at high concentrations, which indicated that it is impacted by the irrigation effluent. This is an indication that there is a probability that irrigation of kraft effluent will impact on the Elands River. However, more work needs to be done to confirm the toxicity of groundwater in the area.

CHAPTER 4

INVESTIGATING THE EFFECTS OF TEXTILE MILL EFFLUENTS ON BAETID MAYFLY NYMPHS OF THE BUFFALO RIVER, EASTERN CAPE

4.1 INTRODUCTION

4.1.1 General

The effective use of freshwater supplies is impaired through water quality deterioration associated with the discharge of untreated or partially treated wastewater into the environment. Continuous discharge of effluents to the environment therefore has long-lasting effects, and may also have social impacts, as downstream users become affected by the poor quality of water. There is a need to safeguard the quality of freshwater supplies and to reduce freshwater use by industry, thus controlling industrial discharges into the environment. It is therefore also important to treat effluent before it is discharged to the environment to minimize the impact. The National Water Act (No. 36 of 1998) is placing considerable responsibility on industries to optimize their water use and to treat their effluents before discharge to the environment [Section 22(2)(e)].

The textile industry is faced with serious problems due to the nature of its effluents. Textile processing plants utilise a wide range of dyes and other chemicals such as acids, salts, detergents, enzymes and bases. Many of these are not retained in the final product and are discharged in the effluent, which ultimately enter the environment. Many of the substances in wastewater are not degradable by self-purification processes and conventional treatment (Davies and Day, 1986). Textile effluent discharged into sewage treatment works causes colour and chemical oxygen demand (COD) problems, and those discharged into the environment introduce a high percentage of colour, COD and salinity (Buckely *et al*., 1990).

In order to place this study in the context of both the Whole Effluent Toxicity (WET) testing approach and international trends in the management of textile effluents, this introductory section begins with a general description of textile effluent, and details the steps in the textile process, with specific reference to a textile factory in the Eastern Cape, South Africa. A general review of investigations into the effects of textile effluents on the freshwater aquatic environment is also included. In this Chapter the use of WET testing to evaluate the response of an indigenous riverine mayfly population to textile effluents is reported.

4.1.2 Textile effluents

General characteristics

The textile industry in South Africa is recognised as one of the largest water users, and produces the highest volume of industrial effluent (Trivedy and Gudekar, 1987; Gravelet-Blondin *et al.*, 1997). Large amounts of water are required for wet processing and the vast quantities of wastewater produced are extremely variable in composition and pollution load (Correia *et al.*, 1994). The pollutants in the wastewater arise from the removal of impurities from the raw material and the residual chemical reagents used for processing. The composition of wastewater from textile plants is complex and varies according to the process used at a plant, as well as depending on the fabric and yarns processed. The strong colour of textile wastewater is the most obvious indicator of water pollution; the degree of colouration dependent on the colour or shade dyed and the type of dye used (Steffen *et al.*, 1993; Carliell *et al.*, 1996). The colour, which is a visible source of pollution, is perceived as harmful. If the colour is not properly dealt with, it can interfere with light penetration, thereby inhibiting or impairing biological processes such as photosynthetic action (Samira and Doma, 1989; Buckley, 1992; Meyer *et al.*, 1992; Lin and Lin, 1993; Gravelet-Blondin *et al.*, 1997).

The removal of dyes from textile effluents is problematic as biological treatment processes are not effective in removing colour (Meyer *et al.*, 1992; Correia *et al.*, 1994; Carliell *et al.*, 1996). Reactive dyes are the most difficult to remove due to their

solubility as they pass through biological sewage treatment systems and enter the receiving water (Lin and Lin, 1993; Carliell *et al*., 1996). Dyes and surfactants predominate in textile effluents, as they are not fully retained in the final product. Azo dyes such as Orange II ($C_{18}H_{11}O_4NaSN_2$) represent the largest group of textile dyes.

The textile effluent contains high but variable concentrations of Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) (Buckley, 1992; Orhon *et al*., 1992; Correia *et al*., 1994). Dissolved Oxygen (DO) is almost zero, Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) are usually high in the effluent. Temperature is relatively high due to hot rinse water. Effluents are highly alkaline with a pH range of 8.2 to 12.2. Chromium is also generated from the chemical material used in the dyeing process (Germirli *et al*., 1990).

Sodium hydroxide is used extensively in the textile industry, resulting in high concentrations of chloride and sodium in the effluent. The discharge of industrial effluents containing sodium hydroxide is problematic since these effluents contribute to the mineral enrichment and increasing salinisation of the receiving waters.

The manufacturing process (raw material and final product)

The production sequence for textiles is shown in Figure 4.1. There are three main processing stages of fibres: fibre pre-treatment**,** dyeing and finishing. The fibre pre-treatment prepares yarns and fabrics for dyeing by removing foreign impurities and assuring good wettability, the required whiteness and high dye intake. There are both dry and wet processing stages.

<u>Fibre pre-treatment</u>

1. Blending and spinning (Steps 1 and 2)

First the raw fibre is sorted and cleaned before being blended as required. Raw cotton and polyester staples are then blended together. The fibres are drawn up into yarn and twisted (spinning). From here, the yarn goes for sizing where enzymes are added to provide protection from abrasion during weaving. The yarn is then washed in hot water counter-current washing machines. Effluent is produced at this stage. The yarn

is sent for weaving, which takes place under controlled high-humidity conditions to minimize the breaking of yarn. At this stage, which is usually a dry process, the yarn is converted into fabric. Desizing follows after weaving, where the sizing agent (enzymes) is removed (Correira *et al*., 1994).

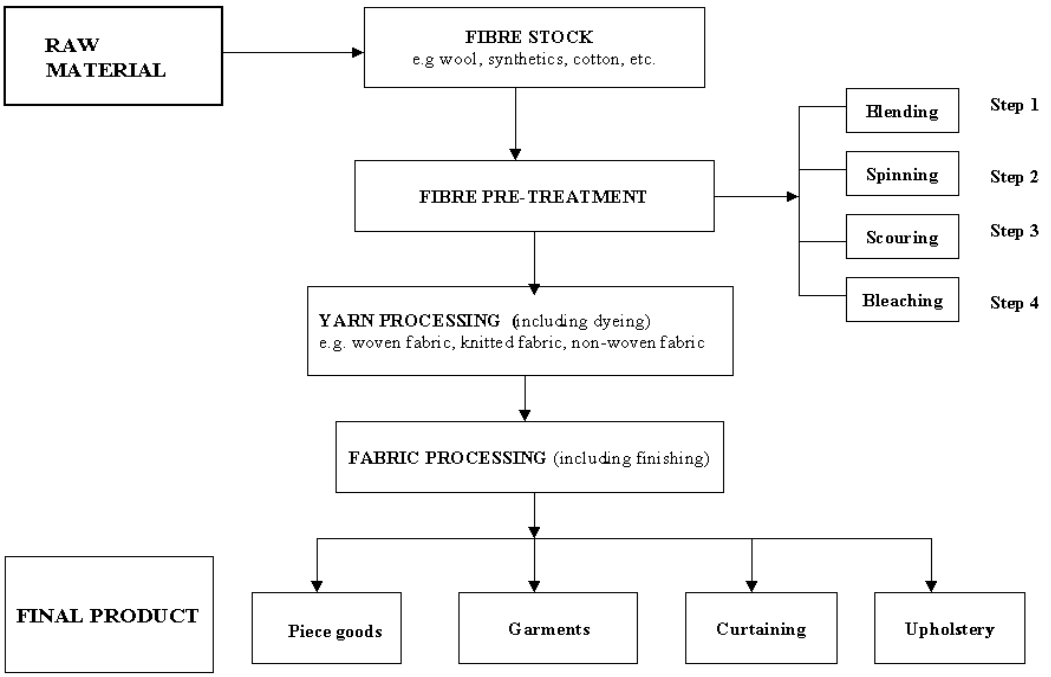

Figure 4.1 A flow chart of the textile production sequence in the textile manufacturing process (Steffen *et al*., 1993).

2. Scouring (Step 3)

The yarn/cotton is scoured to remove natural waxes, spinning oils and other non-cellulosic compounds, using hot alkaline solutions (NaOH) containing detergents or soaps. Raw wool scouring is the highest-polluting process with large volumes of concentrated wastewater being produced. A typical effluent contains wool grease, dirt (from sand, fibre and vegetable matter), and suint salts (salts produced by natural excretions). The COD of the effluent can be as high as 50 000mg/l (Towsend *et al*., 1989). Organochlorine compounds and organophosphates, which are used as parasite control agents by sheep farmers, are found in significant quantities in raw wool scouring effluents (Shaw, 1994). The non-biodegradability of many of the impurities

in scouring wastewater affects the proper operation of biological treatment systems (Correia *et al*., 1994). The disposal of the effluent is mainly by solar evaporation and direct discharge into water resources. Both these methods have become unacceptable due to their environmental impact (Towsend *et al*., 1989).

3. Bleaching (Step 4)

Bleaching aims to remove the natural yellowish colouring of cotton fibres thereby increasing its whiteness. Hydrogen peroxide and sodium hypochlorite are used as oxidizing agents. H_2SO_4, HCl and NaOH are also used during bleaching and final rinsing. After the fabric has been bleached, it is taken for mercerizing where the fabric is treated with NaOH to increase dye-ability and impart sheen. The fabric is then washed with a weak organic acid in order to neutralize the fabric. The effluent produced is highly alkaline, is at high temperatures and has high residual concentrations of sodium hydroxide (Steffen *et al*., 1993). From here, the fabric is sent for either printing or dyeing.

Yarn processing

From bleaching, the fabric goes for dyeing and/or printing. Dyeing may be carried out in a batch or continuous process. Textile processing involves a wide variety of dyes and the fibre concerned determines the selection of the dye. Effluents from batch dyeing of cotton with reactive dyes are usually high in dissolved solids, as the process demands a high concentration of salts and sufficient alkali to raise the pH to 12 (Correia *et al*., 1994). Ninety percent of dyes end up in fabric, with the remaining 10% discharged to waste stream (Porter, 1978 cited by Maguire, 1992). Large quantities of effluent are produced at his stage. Some fabric is sent for printing, where designs are added.

Finishing

Finishing processes involve impregnation of the fabric followed by fixation, heat, and washing to remove residual chemicals. The processes improve the stability of the fabric and impart properties such as stain and shrink resistance, moth and fireproofing. Although the volume of effluents produced is low, they are extremely variable in

composition and can contain toxic organic substances such as ethylchlorophosphates and pentachlorophenols (Correia *et al.* 1994). Dyeing-finishing works are heavy water users and their effluents contain synthetic dyes, surfactants and various additives (Timofeeva, 1991).

Generally, the effluent streams discharged from textile mills include wool scouring effluent, textile soaping effluent, effluents from dyeing, bleaching and acrylic emulsion effluent (Townsend *et al*., 1989). As mentioned in Step 3, effluents from wool scouring contain mainly grease, dirt (sand, fibre and vegetable matter) and suint salts (i.e. salts produced by natural excretions) (Correia *et al*., 1994). Wool scouring produces an effluent considered to be the most polluting of textile effluents (Townsend *et al*., 1989; 1992). Textile soaping effluent produced in the cleaning process after dyeing and printing, contains dissolved and colloidal dyestuffs, detergents and some salts. Polyester/viscose effluent contains both soluble and colloidal dyestuff, acetate, alkali, salts and organic auxiliary chemicals (Townsend *et al.*, 1992).

The production of these strong caustic effluents, with the mills not meeting water quality guidelines, resulted in the initiation by the Water Research Commission (WRC) of investigations into development of technologies. The application of these technologies would alleviate discharge problems (Buckely *et al*., 1990).

The majority of textile effluents are discharged and treated in local sewage treatment works. Treated effluent can be disposed of by irrigation, or discharged directly into a river or sea (DWAF, 1999). Effluent discharged by sewer must meet set standards that comply with the requirements set by the local authority. These requirements in turn must comply with DWAF standards. However, where effluent is discharged directly into the marine environment, the river or discharged for irrigation, the mill must have a license from DWAF as a water user.

Textile effluent treatment

Biological treatment processes such as aerated lagoons and conventional activated sludge processes are frequently used to treat textile effluents and are efficient in the removal of suspended solids and COD, but not colour. Adsorption appears to offer the best prospect for overall treatment and promises also to be effective for the removal of colour (Meyer *et al.*, 1992; Altinbas *et al*., 1995). Davies and Cottingham (1994) found that the visible colour of the textile effluent was reduced as the effluent passed through wetland beds. Ozonation and chlorine were also found to be highly effective in removing colour (Lin and Lin, 1993; Tünay *et al.*, 1996). Although ozone residuals are toxic to aquatic organisms, they are rapidly reduced in wastewater, hence unlikely to be found in the final discharge. For high strength dyes, ozonation is used to remove colour and reduce turbidity, in combination with chemical coagulants such as aluminium sulphate (Lin and Lin, 1993). Chlorine is more effective at lower pH, but there are concerns about the effects of residuals and by-products on the aquatic environment (Nicolaou and Hadjivassilis, 1992). Trivedy and Gudekar (1987) found the water hyacinth to be very efficient in treating textile wastewater; the treatment is attributed to microbial activity.

4.1.3 Effects of textile effluents on aquatic environments

It appears that the effects of textile effluents on aquatic environments have not been well researched. More focus has been placed on effluent treatability. A few studies have indicated that textile effluents have negative environmental impacts. The free chlorine generated during the bleaching process is toxic to microorganisms, which are responsible for the self-cleansing of receiving waters (Davies and Day, 1986; Whitehurst and Lindsey, 1990). Chlorine and chloride have also been found to be acutely toxicity (DiGiano *et al*., 1992; Williams, 1996). Organochlorine compounds and organophosphates used as parasite control agents end up in the effluent, and render it toxic (Shaw, 1994). Unionized ammonia (NH_3) has been reported as a source of toxicity (Doi and Grothe, 1989 cited by Wells *et al*., 1994); Dallas and Day, 1993; DWAF, 1996f). In the studies by Wells *et al.* (1994) and Everitt (1999), zinc was identified as the major contributor to acute *D. pulex* toxicity. Metals present in

effluents are also easily available to the organisms, as they do not easily degrade (Cooper, 1993).

A limited range of studies has recorded investigations of the biological effects of exposure to textile effluents. The colour of the effluent is damaging to the aesthetic nature of the receiving waters, and toxic to aquatic life (Nawar and Doma, 1989; Meyer *et al*., 1992). Dyeing and printing effluents are known to be potent inhibitors of various enzymes, including membrane bound ATPase, an enzyme responsible for the movement of ions across the membrane (Kundus *et al.,* 1992 cited by Chhaya *et al*., 1997). Chhaya *et al*. (1997) found liver shrinkage of the mudskipper *Periophthalmus dipes* after exposure to dyeing effluent. A fish brain study showed a progressive inhibition in the activity of Na^+ and K^+- ATPase, which interfered with the potassium ions influx and sodium ions efflux from the cell. The inhibition of Ca^{2+}-ATPase and Mg^{2+}-ATPase reduced the uptake and transport of Ca^{2+} and Mg^{2+}, which are responsible for muscle contraction (Chhaya *et al*., 1997). Ozoh (1984) found dyeing effluent to be very toxic to *Hippopera nigeriae,* the Nigerian earthworm.

Organics in textile effluent, have high proportions of solids, which can rapidly blanket benthic habitats thus depriving organisms of light and therefore limiting primary production. Organic waste can also reduce DO concentrations in receiving water (Cooper, 1993). Fine particles interfere with the filter-feeding functional group of macroinvertebrates (Mayack and Waterhouse, 1983).

4.1.4 Aims and approaches of the study

This study aims:

- to investigate the potential effects of textile effluent on indigenous riverine mayfly nymphs (Family: Baetidae), from the Buffalo River;
- to investigate the use and practicality of baetids as test organisms for setting water quality guidelines to protect the aquatic ecosystems from adverse effects from pollution;
- to report on the relative toxicity of general and post-irrigation textile effluents; and
- to provide a set of hazard-based guidelines which relate textile effluent toxicity to

river health.

Since the effluent reaches the river via overflow and seepage from the Tailwater Dam, a sub-lethal toxicity test was conducted using effluent from the Tailwater Dam to ascertain its toxicity. The effluent proved not to be acutely toxic. Chapter 4 therefore comprises an evaluation of the toxicity of General Textile Effluent (GTE) from a textile mill in the Eastern Cape, SA, using an acute WET approach; and a preliminary application of a site-specific, hazard-based guideline development procedure. The study involved acute WET toxicity testing during two successive summer periods, as well as during one autumn and winter period.

4.2 THE TEXTILE INDUSTRY IN SOUTH AFRICA

4.2.1 General

The South African textile industry was established in the first half of the twentieth century, and by 1939 was providing 3 500 jobs (Textile Federation, 1994 cited by Gilfillan, 1997). By 1960, there were about 65 textile factories producing knitted fabrics, cotton-based yarns and woven fabrics in South Africa, indicating a fast growth in the industry. The economic instability in South Africa has had an impact, retarding the trend of growth, and in 1996, there were approximately 70 textile factories registered with the Textile Federation (Gilfillan, 1997).

The South African textile industry is the sixth largest employer in the country (Gravelet-Blondin *et al.*, 1997), and had fixed assets worth over R 2 billion in 1996, with R 7.9 billion in sales per year (Keller, 1996 cited by Gilfillan, 1997). The introduction of environmental performance indicators such as International Organisation of Standards (ISO) 14 000, has made it difficult for the industry to export unless textiles are manufactured in accordance with environmental legislation (Gravelet-Blondin *et al.*, 1997). South Africa is lagging behind in the implementation of pre-treatment technology, as the relevant government departments have not been enforcing compliance. With the new legislation (particularly the National Water Act (NWA) No. 36 of 1998) in place, textile industries are now forced to look for cost-

effective methods for treating their effluent. Pre-treatment of the effluent may ensure that discharge to municipal sewers is of a fair effluent quality, which may result in reduced tariffs (Gilfillan *et al.*, 1997).

In South Africa, the textile industry generally consumes large quantities of water and produces large volumes of effluent. About 70 to 80 % of the intake is discharged as textile effluent (Steffen *et al*., 1993). Most of the textile factories in South Africa are poorly equipped to deal with modern discharge limits, particularly with regard to dissolved solids, pH, ionic salts, colour and heavy metals, as design specifications did not take account of environmental considerations (Steffen *et al*., 1993). This has resulted in textile effluents impacting heavily on our water resources, thus affecting the quality of the receiving water.

Sections 21 (a) and (f), of the National Water Act (No. 36 of 1998) require that water use be licensed, unless it is listed in Schedule 1 (NWA, 1998, Sections 4(1) and 22(1)(a)(i)). Schedule 1 lists the water users that are exempted from licensing and are issued with the General Authorization. The General Authorization is blanket permission for water users to either abstract, store water from a water resource, to irrigate any land with wastewater or to discharge wastewater into a water resource. It is granted only if the water quality aspect of the activity is within the limits and conditions set out in the authorization. The NWA also requires wastewater to be returned to the source, but the wastewater must meet minimum quality standards for effluents. The majority of textile industries discharge to sewer, and therefore their effluents must comply with standards set by Local Authorities as well as standards set by the DWAF. The effluent quality is specified in General Authorization document (DWAF, 1999). Due to the variable and complex nature of textile effluents, biological treatment does not sufficiently treat the effluents, resulting in colour and substances such as solvents and salts passing through the works and into the aquatic environment (Gravelet-Blondin *et al*., 1997).

4.2.2 Case study: A textile factory in the Eastern Cape

The textile factory used for this study is one of the largest industries in the Eastern Cape. It is unique in that the effluent produced is not discharged directly into the river system, but is used mainly for irrigation. The rest of the effluent is discharged into the Sewage Treatment Works. The mill receives good quality water from the Rooikrantz Dam (DWAF, unpubl. data). About 3.5Ml/d of combined effluent are produced, currently 0.4 Ml/d of this effluent is discharged into the sewer; 0.5Ml/d is sent to evaporation ponds and the rest is used to irrigate grass pastures.

The plant produces three types of effluents (Figure 4.2):

- domestic effluent which goes to the Zwelitsha Sewage Treatment Works;
- high salinity effluent mainly from the scouring process, which goes to a series of caustic evaporation ponds; and
- the main factory effluent, which is used for irrigation.

Flow of effluent from the factory

A detailed diagram of the flow of textile effluent from the factory is shown in Figure 4.2. From the plant, the untreated main factory effluent goes into settling tanks, and overflows via a channel into a distribution dam. From this dam, the effluent goes through a splitter-box, which directs the effluent either to the irrigation canals, or to a balancing dam with a 14 day-retention period. Generally, the effluent is channelled directly through the irrigation canals for flood irrigation. Some of the effluent channelled to the balancing dam is sent back to factory for caustic recovery, and the rest is used for spray irrigation during the day. Any run-off from irrigation and overflow collects via the Mlakalaka stream into the Tailwater Dam. The effluent from the Tailwater Dam is spray-irrigated on kikuyu to reduce the dam volume, thus preventing overflow down the Mlakalaka stream and into the Buffalo River. However, the effluent seeps through the dam wall, running down the stream and into the Buffalo River. During irrigation, evaporation takes place and the remaining water, which contains high salt loads, returns via surface flows or groundwater to the river.

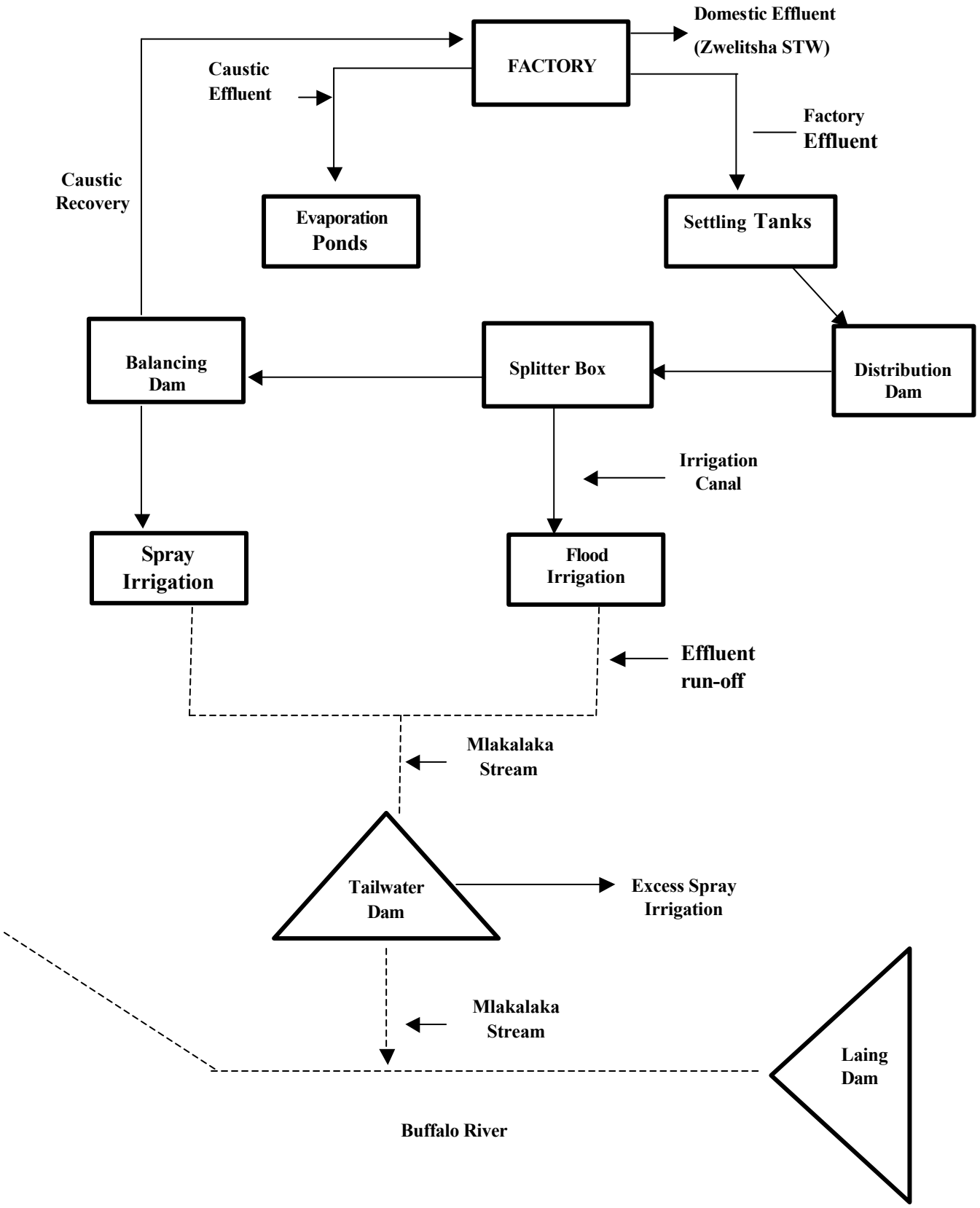

Figure 4.2 Layout of effluent streams and irrigation scheme of a textile factory in the Eastern Cape (modified from Bruinette *et al.*, 1997).

The textile factory occasionally discharges a coloured effluent into the Buffalo River via overflows into the Mlakalaka stream. There is also constant seepage below the dam. Together with the King William's Town Tannery, King William's Town and Zwelitsha Sewage Treatment Works, the textile factory contributes to a salt load increase in Laing Dam (Buckely *et al.*, 1983; O'Keeffe *et al*., 1996), situated about 7km downstream of the factory (Figure 4.3).

4.3 STUDY SITE

4.3.1 Introduction

Test organisms and test water for toxicity tests were collected from the Buffalo River. The site for the collection of test organisms was selected because of its position in relation to point or non-point sources in the catchment (Figure 4.3), i.e. good quality water, test water and test organisms from an upstream unimpacted site, resulting in test organisms not pre-exposed to pollutants or effluent. The site was also selected as riffle-dwelling test organisms were present in abundance.

4.3.2 Buffalo River

General description of the Buffalo River catchment

The Buffalo River provides water to a rapidly growing population in the areas around East London and King William's Town, despite being highly impacted by both point and non-point pollution sources. Laing Dam (Figure 4.3), the largest impoundment on the Buffalo River, receives treated effluent from the Ilitha, Breidbach and Bisho Sewage Treatment Works via the Yellowoods River. The dam acts as a large settling pond and nutrient levels downstream of the dam are considerably reduced (O'Keeffe, 1989; Palmer and O'Keeffe, 1990a; Dallas and Day, 1993). Continuous abstraction along the Buffalo River for urban-industrial use and irrigation keeps flow rates low during dry seasons. Streams such as the Shangani, Tindeli and Sitotana, which collect water run-off and wastewater from sewer pipe-burst and stormwater drainage areas in the Mdantsane Township (outside East London), discharge into the lower reaches of the river. However, this problem has recently been drastically reduced by good

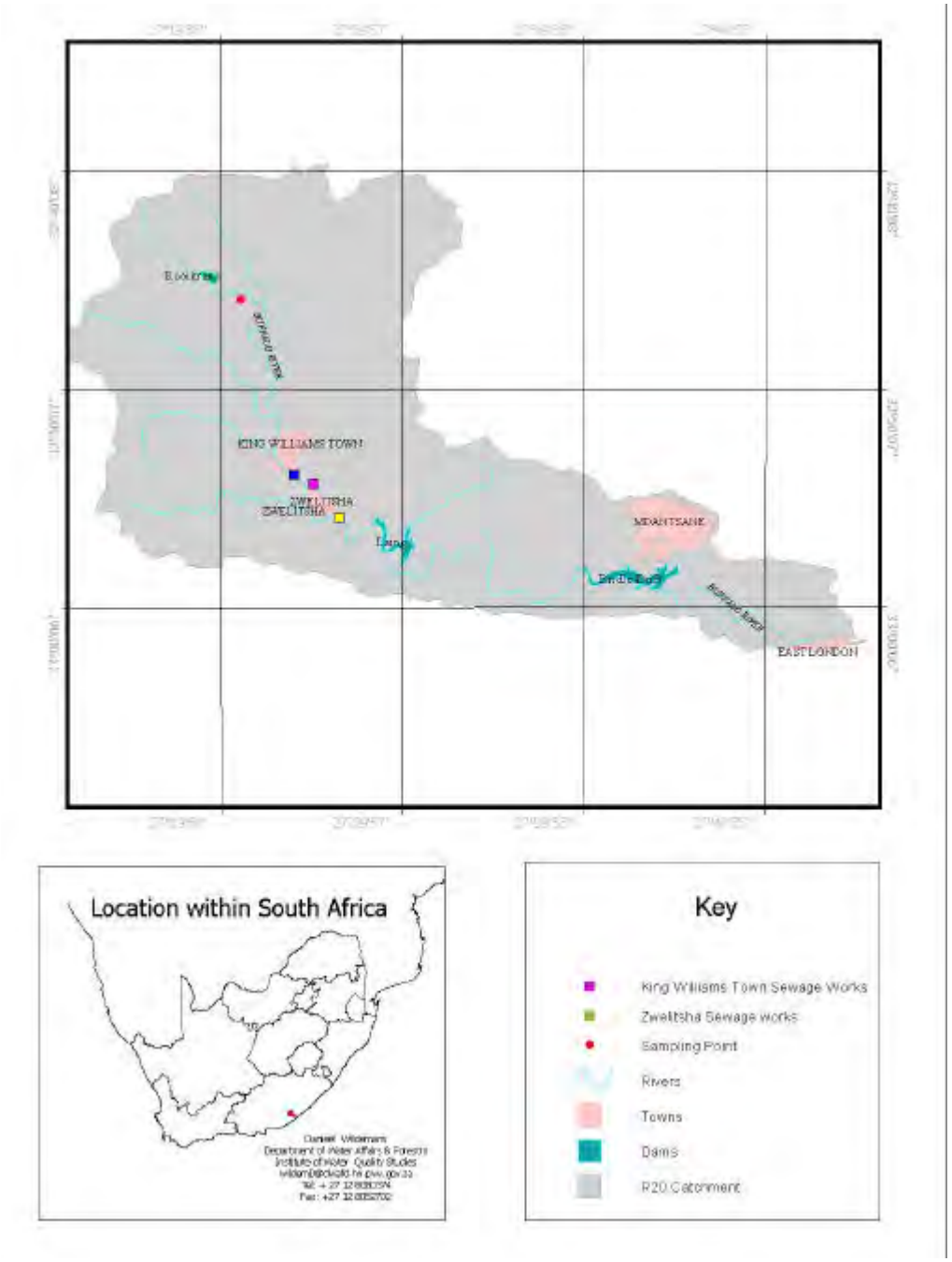

Figure 4.3 Map of the Buffalo River showing the textile factory and sampling site for the study. The position of King William's Town and Zwelitsha Sewage Treatment Works are shown.

sampling site for the study. The position of King William's Town and Zwelitsha management by the East London Municipality. The run-off contributes to high faecal coliform counts, and eutrophication by blue-green algal blooms in the Bridledrift Dam (Hart, 1982; Selkirk and Hart, 1984; DWAF, unpubl. data).

Topography and geology

The Buffalo River is short (140km), steep and deeply incised along most of its course to the estuary. It runs in a south-easterly direction and drains into the estuary and then into the sea at East London (Figure 4.3). The river flows through several vegetation types. The geology of the catchment consists mainly of sedimentary rocks of the Lower Beaufort Series of the Karoo System, with a few dolerite outcrops (Hart, 1982; Stone, 1982).

Vegetation

The natural historical vegetation of the Buffalo River catchment consists of five main types: small areas of False Macchia at the summit of the Amatole Mountains, Yellowood forest on the slopes of the mountains, False Thornveld (dominated by grassland and *Acacia karoo*) which covers the middle catchment from below Rooikrantz to Bridledrift Dams, Valley Bushveld in the immediate river valley, and the Coastal Forest and Thornveld in the lower reaches (O'Keeffe *et al*., 1996). Most of the vegetation has been destroyed, leaving only forests in the upper and lower parts of the catchment, which cover an area of approximately 140 km^2 (O'Keeffe *et al*., 1996).

Land-use

The upper area of the river catchment is important for timber, nature conservation and recreation (hiking and angling), and generates 42% of the total run-off of the river (O'Keeffe, 1989). Land-use in the upper middle area of the river is trout fishing and agriculture (mainly grazing and irrigated market gardening). Tannery and textile industries are situated in the middle reaches of the Buffalo River, and their effluents irrigate the grass pastures. Although the effluent is used for irrigation, the run-off and seepage enter the Buffalo River. These effluents contribute to the increase in natural salinisation in the Buffalo River, as they contain high levels of TDS, chloride and

sodium (DWAF, unpubl. data). Two Sewage Treatment Works, King William's Town and Zwelitsha, are also situated in the middle reaches, and are discharging their treated effluents into the Buffalo River thus contributing to increased phosphate levels. Another two Sewage Treatment Works, Mdantsane and Potsdam, are situated in the lower middle reaches of the Buffalo River, and their combined effluents are also discharged into the river below the Bridledrift Dam. The lower reaches are mainly used for grazing and agriculture. Bridledrift Dam is also used for recreation such as fishing and boating.

4.3.3 Water quality

The Buffalo River headwater stream is typical of a mountain stream, with turbulent, clear, good quality water that is free of silt and rich in oxygen. The upper middle reaches of the river, where the sampling site for this study is located, has low salinity levels compared to the downstream stretches of the river. Generally, pH is normal and TDS is relatively low, although there is a progressive increase of TDS/salinity as the river flows towards the estuary, due to saline effluents that are discharged into the river (Selkirk and Hart, 1984; DWAF, unpubl. data). Nutrient levels are largely insignificant (based on the results of Table 4.2). Generally, the water quality appears to be good in the upper middle reaches of the river.

From the middle reaches down the river, the water quality deteriorates. The major water quality problems are salinisation and eutrophication in the Laing and Bridle Drift Dams, and faecal contamination in Bridle Drift Dam (Selkirk and Hart, 1984; DWAF, unpubl. data). Recently (Oct/Nov 1999), Laing Dam has been experiencing blue-green algal blooms, which may be due to the severe drought and very hot weather (Kooverji, pers. comm.).

4.4 MATERIALS AND METHODS

The methods used are detailed in Chapter 2, and only those specific to the textile mill effluent experiments conducted in the Zwelitsha laboratory are detailed in this section.

4.4.1 Collection of test organisms and experimental medium

A preliminary investigation of the Buffalo River revealed that baetid nymphs were in abundance in an unimpacted area upstream of King William's Town. Their absence in the polluted downstream section of the river could indicate their sensitivity to effluents released downstream. Although present throughout the year, the abundance of baetids appeared flow-related. Field investigation during winter months showed very few baetids present, probably related to reductions of flow. Reduced flow conditions can induce organisms to release their hold on the substrate and swim into the water column (Minshall and Winger, 1968 cited by Corkum, 1977). During some summer months, mayflies were swept away by heavy flows, which would be expected, as baetids rely on claws and swimming to resist currents (Hynes, 1960).

Baetid (Ephemeroptera) nymphs were collected from shallow, rocky, riffle areas in the Buffalo River downstream of the Rooikrantz Dam (Figures 4.3 and 4.4). This site was chosen as it was considered "unpolluted" and not impacted by any effluent or point source discharges. It is also one of the sites used by Palmer and O'Keeffe (1990a,b) during the Buffalo River Programme conducted at the IWR from 1986 to 1988. The nymphs of baetid mayflies were selected as test organisms because other researchers and institutions such as US EPA, American Standards of Testing Materials (ASTM) (Persoone and Janssen, 1993), and the IWR (Palmer *et al*., 1996; Williams, 1996; Binder, 1999) used them routinely, and they were in abundance.

After collection from the test site (Figure 4.4), the test organisms were transported a distance of about 20 km to the Zwelitsha laboratory for sorting. As baetids are extremely sensitive to handling and can be easily damaged (Palmer *et al*., 1996; Williams, 1996), handling was kept to a minimum. After sorting, about 30 to 40 organisms were placed in each artificial stream system. Since the baetids from the sampling site appeared to be a mixed population, and it is not possible to speciate baetids live, a great effort was made to select similar-looking organisms so as to increase the probability of using a greater percentage of the same species. The nymphs with wing-buds were not used, as they would probably emerge during the experiment.

Buffalo River water was collected in 25 litre plastic containers from the same site where the test organisms were collected. River water was used as test diluent as well as control medium. It was analyzed by IWQS before the start of the project to ascertain its suitability for use as test medium. Physico-chemical analysis results indicated good water quality (Section 4.5.1, Table 4.2).

Figure 4.4 Sampling and collection site in the Buffalo River during low flows.

The textile effluent used as the toxicant was collected from two points: i) the settling tanks before irrigation (i.e. General Textile Effluent), and ii) post-irrigation from the Tailwater Dam weir after irrigation. General effluent is therefore the effluent directly from the mill, excluding caustic effluent and sewage effluents. Post-Irrigation Textile Effluent is the seepage and run-off from the irrigated land that collects in a holding dam, the Tailwater Dam. The results of chemical analyses by IWQS are presented in Section 4.5.1, Table 4.2. Grab samples were taken in 25 litre plastic containers. Grab samples were preferred for acute toxicity testing since the effluent was highly variable (Burton *et al*., 1996).

4.4.2 Experimental approach

Laboratory design

Twelve recirculating artificial streams, the *channels* (Figure 2.1), were set up at the Zwelitsha Scientific Services laboratory near King William's Town, which is under the control of the DWAF in the Eastern Cape region. Laboratory temperature was controlled with the use of a Panasonic room air-conditioner, Model CW-A90FN, and maintained between 16°C and 22°C (mean = 19.4°C, standard deviation ± 2.3°C). Maintaining the laboratory temperature at a smaller range was difficult due to the fluctuation in ambient temperatures. Lighting was maintained at a 12:12 hour light:dark cycle with OSRAM biolux tubes providing wavelengths of light similar to sunlight (Palmer *et al*., 1996).

Experimental stream systems and experimental procedure

The channel stream systems used for toxicity tests are described in Chapter 2. After an acclimation period of 36 hrs, 96 hr acute and 7 day sub-chronic toxicity tests were conducted. The Buffalo River water was used as test water, the textile effluent as toxicant, and the baetids as test organisms. For general experimental procedure refer to Chapter 2. Test organisms in channels were exposed to increasing percentages of textile effluent (Table 4.1) in a regression design (Section 2.5), with one channel used as a control. During Experiment 7 (a 7 day sub-chronic toxicity test), the test medium was replaced with freshly prepared test medium after 96 hours to reduce the build-up of toxins and metabolites, such as ammonia, in the water (Coler and Rockwood, 1989). All the preserved test organisms were sent to the IWR at Rhodes University for identification by Mr. KM Soxujwa, as it was difficult to identify the organisms before the start of the experiment.

Water quality analyses

The whole effluent was chemically analysed by the IWQS at the start and finish of each experiment to provide information on chemical composition, and to determine the variability in the measured variables over time, and between individual experiments. Daily measurements of pH, temperature and EC were routinely taken in

each experimental channel. The Amel digital conductivity meter (model 160, graphite electrode model 193) was used for EC measurements, and the Knicks calimatic pH meter 601, for pH readings.

TABLE 4.1
PERCENTAGE CONCENTRATIONS OF TEXTILE EFFLUENT USED FOR ACUTE (96 HOUR) AND SUB-CHRONIC (7 DAY) TOXICITY TESTING WITH BUFFALO RIVER WATER AS DILUENT AND CONTROL.

Experiment number	Type of Experiment	Type of Effluent	Effluent concentration (%)	Starting date
1	Acute	General Textile Effluent (GTE)	1,3,5,10,20,30,40,50,60,75,100	24-11-1997
2			1,3,10,30,40,50,100	01-05-1998
3			1,3,10,15,20,25,30,50	07-05-1998
4			1,3,5,10,15,20,25,30,50	11–05-1998
5			0.5,1,3,5,10,15	15-05-1998
6	Acute	Post-Irrigation Textile Effluent (PITE)	1,3,10,15,20,25,30,50	21-05-1998
7	Sub-chronic		1,3,5,10,20,30,50,60,75,100	02-06-1998
8	Acute	General Textile Effluent (GTE)	1,3,5,10,15,20,25,30,50,100	11-11-1998

Data analysis

The experiments were set up using a regression design with one channel at each dilution, plus a control. The Probit and Trimmed Spearman-Karber methods were used to calculate LC50 values, as described in Chapter 2. The Probit method was preferred as it also provides LC1 and LC5 values. These values were used to derive the Acute Effect Values (AEV) (Section 2.9.4) (see DWAF (1996f) for methods). The AEV, LC1, LC5, and the associated 95% confidence limits were used to apply the hazard-based approach of Palmer and Scherman (in press). This approach links toxicity test results to river health classification (DWAF, 1999a; Kleynhans, 1999).

4.5 RESULTS

In this the study attention was paid to the toxicity of two textile effluents: (i) general textile effluent (GTE), and (ii) post-irrigation effluent (PITE). Six 96 hr acute tests were conducted using GTE, and 1 acute and sub-chronic test using PITE. The PITE effluent reaches the river via an overflow from the Tailwater Dam weir down the Mlakalaka stream.

4.5.1 Chemical composition of Buffalo River water and textile effluent

Table 4.2 presents a physico-chemical analysis of Buffalo River water, collected at the sampling site used for this study in 1997, at the start of the study, and textile effluents (GTE and PITE), for 8 samples collected in 1997 and 1998. Chemical analysis was conducted by IWQS. Effluent data are presented in ranges. A comparison of all 100% textile effluent samples is presented in Tables 4.12 and 4.13. These tables also give a total picture of the variability of textile effluent batches.

As shown in Table 4.2, salts are a major component of the textile effluent, particularly sodium ions. This is to be expected as NaCl is used heavily during fibre processing. Total Dissolved Solids (TDS) and electrical conductivity (EC) were about 15 and 20 times higher than the receiving water respectively. According to the South African Water Quality Guidelines (SAWQG) for Aquatic Ecosystems, TDS should change by not more than 15% from the normal cycles of the water body under unimpacted conditions (DWAF, 1996f). The PITE had higher chlorides, calcium and phosphates levels than the GTE. Higher chloride levels could be due to accumulation on the soil surface during irrigation, followed by washed-off into the dam. 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, 1999b). This could be expected, as the fabric goes through copper roler printers during printing. The effluent was characterised by a blue-black colour, soapy to touch and smelt of ammonia.

TABLE 4.2
COMPARATIVE PHYSICO-CHEMICAL CONSTITUENTS OF RIVER WATER AND TEXTILE EFFLUENT (EXPRESSED IN MG/L), EASTERN CAPE FACTORY SAMPLES COLLECTED FROM THE GENERAL EFFLUENT STREAM (1997 AND 1998) AND EFFLUENT FROM TH E TAILWATER DAM (POST-IRRIGATION EFFLUENT) (1998) FOR ACUTE (96 HR) AND SUB-CHRONIC (7 DAY) TOXICITY TESTING. DATA IS PRESENTED AS RANGES AND WAS ANALYZED BY IWQS. * BELOW DETECTION LIMIT

Parameter (mg/l)	Buffalo River	General Textile Effluent (GTE)	Post-Irrigation Textile Effluent (PITE)
EC (mS/m)	15.2	183.0 - 247.0	223.0 - 256.0
TDS	87	1607.0 - 2173.0	2085.0 - 2147.0
pH	7.7	8.2 - 8.6	7.8 - 8.9
SO^{2-}_4	35.0	227.0 - 273.0	225.0 - 262.0
TAL	7.0	968.0 - 829.0	829.0
Cl^-	13.0	114.0 - 144.0	332.0 - 341.0
Ca^{2+}	11.0	3.0 - 6.0	17.0 - 19.0
K^+	4.0	16.5 - 33.3	2.0 - 21.0
Na^+	1.1	485.0 - 631.0	590.0 - 616.0
NH_4^+–N	8.0	2.9 - 20.8	0.3-0.7
NO_3^-+NO_2^--N	<0.04*	0.30 -1.30	0.10 - 0.30
PO_4^{3-}-P	<0.04*	<0.005*	1.80 -1.90
B	0.03	<0.005*	0.10
Cr^{2-}	<0.005*	<0.005*	<0.005
Cu^{2+}	<0.005*	<0.005 - 0.40	<0.005 - 0.30
Fe^{3+}	<0.005*	0.90 -1.10	0.70 - 0.80
Mn^{2+}	<0.04	<0.04 - 0.30	<0.04 - 0.30
Zn^{2+}	<0.005	0.10-1.70	<0.005 - 1.60

4.5.2 Comparison of Probit and Trimmed Spearman Karber (TSK) LC50 values

A total of seven acute WET tests and one sub-chronic WET test were conducted using textile effluent as the toxicant, and baetids as test organisms. The 96 hr LC50 values and their 95% confidence limits were calculated using both Probit analysis and the Trimmed Spearman-Karber (TSK) method (Chapter 2), and are graphically presented as cumulative mortality and concentration-response curves (Figures 4.5 - 4.20). The Probit method was preferred as it provides LC1 and LC5 values, which were used in the calculation of the Acute Effect Value (AEV) for hazard-based assessments. A

detailed account of the results for each Probit and TSK analysis is provided in Appendix B. Table 4.3 shows LC50 values from Probit and TSK analyses together with 95% confidence limits, for individual experiments, during WET testing. The table shows that for Experiments 5, 6 and 7, both Probit and TSK analyses were not appropriate, and for Experiment 2, only TSK analysis was appropriate. The chi-square values were similar and low for all GTE batches, indicating that there were insignificant differences between observed and expected values and that the Probit method was appropriate for data analysis.

TABLE 4.3
LC50 VALUES AND CONFIDENCE LIMITS FOR BAETIDS IN TEXTILE EFFLUENTS. 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; c^2 = CHI-SQUARE)

Experiment number	Type of Effluent	Probit				Trimmed Spearman-Karber			
		LC50	95% LCL	95% UCL	c^2	LC50	95% LCL	95% UCL	Trim %
1	General Textile Effluent (GTE)	25	21	28	7	24	19	29	0
2		PROBIT METHOD NOT APPROPRIATE				7	4	12	28
3		11	9	13	8	9	8	12	4
4		6	4	8	8	5	4	7	8
5		PROBIT PROGRAM NOT APPROPRIATE				SPEARMAN-KARBER METHOD NOT APPROPRIATE			
6	Post-Irrigation Textile Effluent (PITE)								
7									
8	GTE	16	14	17	9	14	12	17	0

These results indicate that it is not possible to quantitatively compare mayfly responses to both GTE and PITE, but the PITE was clearly less toxic, with very low responses even at high effluent concentrations (Figures 4.15 - 4.18). 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).

4.5.3 Toxicity test results and associated effluent chemistry

Figures 4.5 - 4.20 show percentage cumulative mortality and concentration-response curves of General Textile Effluent (GTE) for baetids, during the acute 96 hr and sub-chronic 7 day experiments, and Tables 4.4 - 4.11 present ranges of the daily measurements during the experiments using GTE and PITE. The full physico-chemical results for individual experiments are presented in Tables 4.12 and 4.13.

Experiment 1 General Textile Effluent (24-11-1997)

Description: *GTE Exp 1*

Exposure of baetids to GTE for 96 hrs was conducted using a range of effluent concentrations (Table 4.1). There was a power failure at the start of the acclimation period, stopping water recirculation for a period of about 30 minutes. This did not appear to affect test organisms, and the acclimation mortality was 6%.

Water quality

Table 4.4 shows the ranges of daily measured variables per channel during Experiment 1. Higher effluent concentrations reached 100% responses within the first 12 hrs. Only one reading for each variable was therefore taken. Channels with high effluent concentrations were very dark in colour, and torch light had to be used for mortality checks.

TABLE 4.4
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 1. *INDICATES THAT ONLY ONE READING TAKEN AS 100% MORTALITY WAS OBSERVED WITHIN 12 HRS.

Para-meter (mg/l)	% Effluent concentration											
	0	**1**	**3**	**5**	**10**	**20**	**30**	**40**	**50**	**60**	**75**	**100**
EC (mS/m)	41.8-44.3	42.4-45.7	43.9-46.6	47.0-50.6	54.5-57.5	66.9-70.8	79.1-83.0	92.3-98.1	104.4 –111.1	128*	150 *	193*
pH	8.3-8.4	8.3-8.4	8.3-8.5	8.4-8.5	8.5-8.6	8.5-8.7	8.7-8.9	8.7-9.3	8.7-9.5	9.6*	9.9 *	10.5*
Temp. (°C)	16.0-19.0	16.0-18.8	16.1-19.0	16.1-19.0	16.0-19.0	16.2-19.1	16.1-19.0	16.2-19.1	16.2-19.5	19.2 *	19. 6*	19*

This batch of effluent was characterized by a blue-black colour. The pH was alkaline, and it never fluctuated by more than 0.3 pH units at low concentrations. The pH variation become evident at high concentrations, as the pH increased with increasing effluent concentrations, and was above the General standard of 9.5 pH units (DWAF, 1991). The channel temperatures fluctuated between 16.0 and19.5°C, and laboratory temperatures ranged from 16.0 to 22°C. The laboratory temperature was high on the first day of the experiment, but stabilised thereafter to between 16.0 and 18.0°C. This shows a fluctuation of ±2°C. EC was less than 200 mS/m, which is the limit set for effluent irrigation without a licence (DWAF, 1999).

Toxicity results

Mortality in the control was 6%, and mortalities for 1%, 3% and 5% were similar to the control, i.e. they were below 10%. All organisms at 75% and 100% effluent concentrations died within the first 12 hours of the experiment. The Probit LC50 value was calculated at 25% effluent concentration, with a narrow range of confidence limits (Table 4.3). The Trimmed Spearman-Karber LC50 was at 24% effluent concentration. The concentration-response curve showed that at 50% effluent concentration, all organisms died within the 24hrs (Figure 4.6). Figure 4.5 shows 50, 60, 75 and 100% effluent concentrations causing 100% mortality.

Experiment 2 General Textile Effluent (01-05-1998)

Description: *GTE Exp 2*

Baetids were exposed to a range of GTE concentrations for 96 hrs (Table 4.1). Acclimation mortality was zero.

Water quality

Table 4.5 shows the ranges of daily measured variables during Experiment 2. Higher effluent concentrations reached 100% responses within the first 12 hrs, as a result only one reading in each variable is presented. High concentrations were very dark in colour, and torch light had to be used for mortality checks. Laboratory temperature

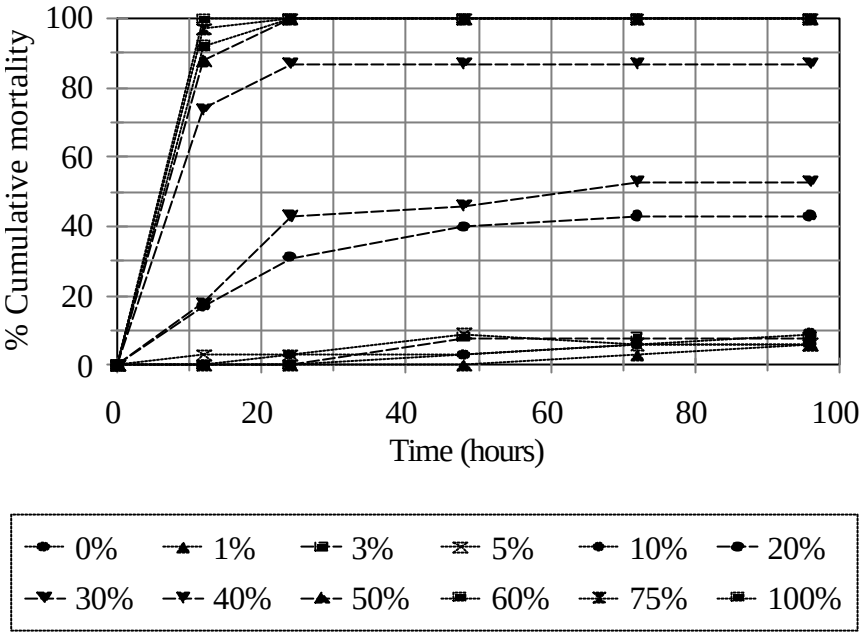

Figure 4.5 Experiment 1: The percentage cumulative mortality of baetids over 96 hrs, after exposure to General Textile effluent at a range of effluent concentrations. The diluent was Buffalo River water.

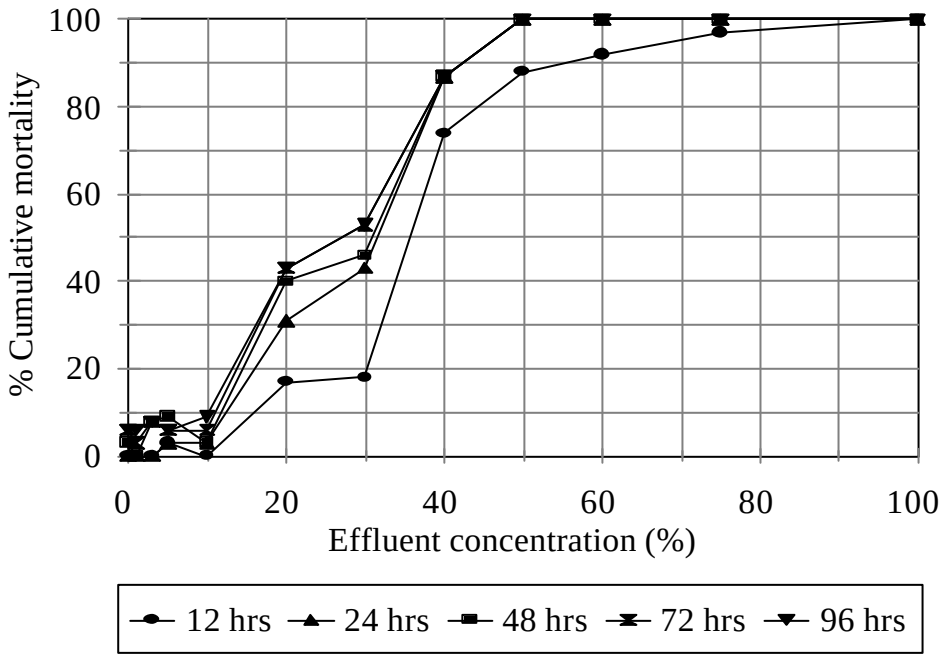

Figure 4.6 Experiment 1: Concentration-response curve for baetids exposed to General Textile Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Buffalo River water.

fluctuated between 17 and 21°C. The full physico-chemical analysis of the effluent used is presented in Section 4.5.4, Table 4.12.

TABLE 4.5
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 2. * INDICATES THAT ONLY ONE READING TAKEN AS 100% MORTALITY WAS OBSERVED WITHIN 12 HRS.

Para-meter (mg/l)	% Effluent concentration							
	0	**1**	**3**	**10**	**30**	**40**	**50**	**100**
EC (mS/m)	19.7-23.0	21.2-25.0	25.3-28.0	39.2-45.0	81.7-92.0	97.8-116.5	192*	283.5*
pH	7.9-8.3	7.9-8.2	8.0-8.2	8.0-9.5	9.37-10.5	9.6-10.6	10.5*	11.3*
Temp. (°C)	16.0-19.0	16.2-19.5	16.2-20.0	16.0-19.5	16.0-20.0	16.1-20.0	20*	20*

This batch of effluent was characterized by a blue-black colour and high turbidity, which reduced visibility at higher effluent concentrations. Effluent was alkaline, with pH above the General Standard limit of 9.5 pH units (DWAF, 1991), and increased with increasing effluent concentration. The channel temperatures fluctuated between 16.0 and 20.0°C, with a variation of ± 4°C over 96 hrs. EC was higher than the recommended limit for irrigation of effluent (DWAF, 1999b).

Toxicity results

This batch of effluent appeared to be more toxic than the effluent sample used in Experiment 1. At 50% and 100% effluent concentrations, all test organisms died within the first 12 hrs of the start of the experiment (Figure 4.7). Mortalities at 1% and 3% effluent concentrations were almost 5 times higher than of the same concentrations in Experiment 1, although the batch was also a GTE. The concentration-response curve (Figure 4.8) showed that 50% mortality was reached at 10% effluent concentration within 48 hrs. The Probit method could not calculate the LC50, as mortality data did not meet the distribution properties required by Probit analysis. However, the Spearman-Karber analysis showed LC50 at 7% effluent concentration, with a wide range of 95% confidence limit and a high percentage trim

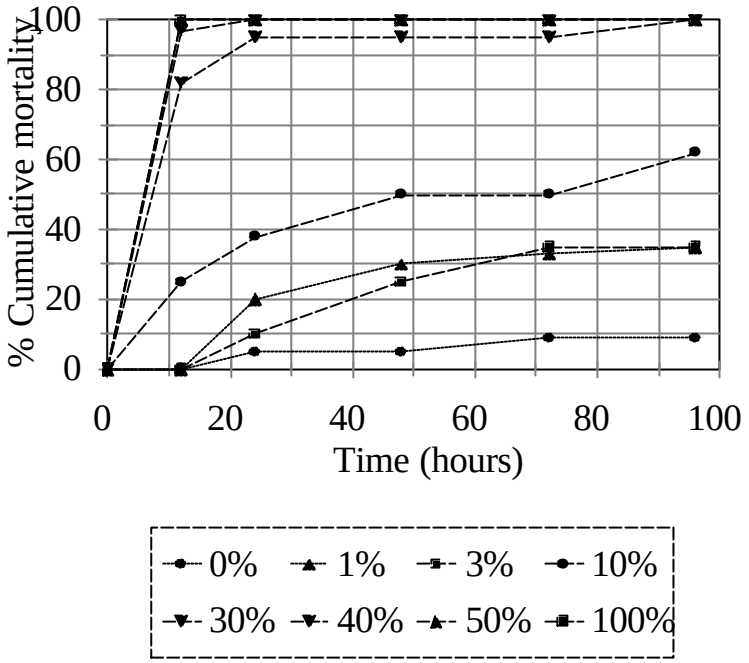

Figure 4.7 Experiment 2: The percentage cumulative mortality of baetids over 96 hrs, after exposure to General Textile effluent at a range of effluent concentrations. The diluent was Buffalo River water.

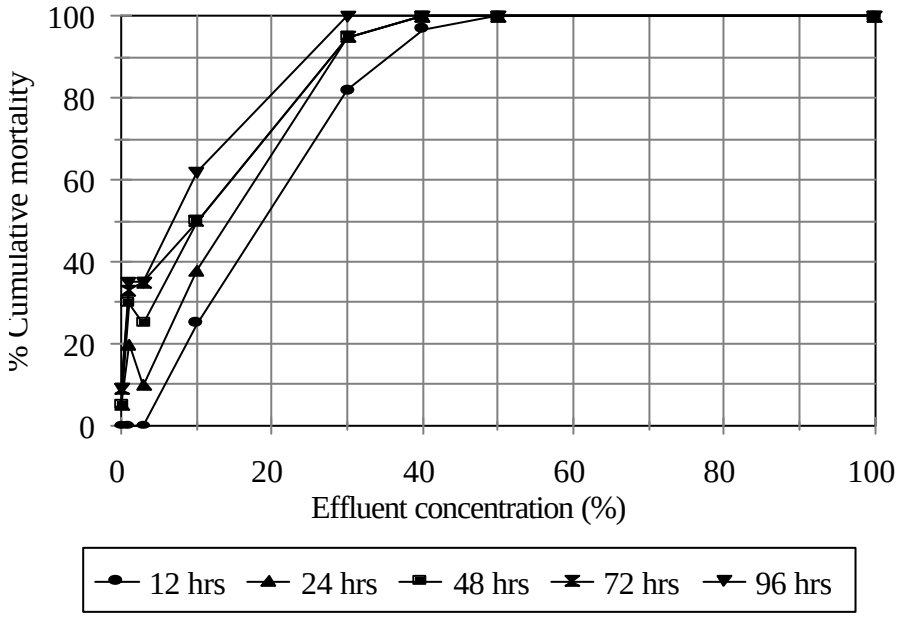

Figure 4.8 Experiment 2: Concentration-response curve for baetids exposed to General Textile Effluent at a range of effluent concentrations over various time periods (12-96hrs). The diluent was Buffalo River water.

(Table 4.3). The high percentage trim i.e. 28%, suggests that the mortality data does not fit this model very well, and that the LC50 value is not very reliable.

Experiment 3 General Textile Effluent (07-05-1998)

Description: *GTE Exp 3*

Baetids were exposed to a range of effluent concentrations (1-50%) for 96 hrs, as Experiments 1 and 2 showed 100% response within the first 12 hrs (Table 4.1). The acclimation mortality was 3%. Daily measured variables are shown in Table 4.6. Responses of the organisms are shown in Figures 4.9 and 4.10. In this experiment *Afroptilum parvum* was the dominating species at 68% (Table 4.17).

Water quality

Table 4.6 presents ranges of daily measured variables for GTE, per concentration.

TABLE 4.6
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 3

Para-meter (mg/l)	% Effluent concentration								
	0	**1**	**3**	**10**	**15**	**20**	**25**	**30**	**50**
EC (mS/m)	15.2-18.9	16.4-20.1	19.1-22.9	28.3-34.2	35.0-42.0	39.5-50.3	47.4-57.3	60.4-63.0	89.2-93.9
pH	7.3-7.7	7.8-8.8	7.9-8.3	8.3-9.2	8.5-9.6	8.5-9.7	8.6-9.8	8.7-9.9	9.3-10.2
Temp. (°C)	14.0-19.5	13.5-19.0	13.5-21.0	13.5-21.0	13.5-21.0	13.5-21.0	13.5-21.0	18.8-19.2	18.8-19.5

This effluent batch was characterized by a blue-black colour. Its turbidity was high.

The EC was within the recommended standards for irrigation using an industrial effluent (DWAF, 1999b). The pH of the effluent was alkaline, and showed an increase with increasing concentration. The channel temperatures fluctuated between 13.5 and 21°C (Table 4.6), i.e. ± 7°C fluctuation. This was higher than the recommended range and could have contributed to higher mortalities, except that the control mortality was low.

Toxicity results

Control mortality was 4% after 96 hours. Low effluent concentrations (1 and 3%) showed low mortality (8%). Figure 4.9 shows that 30 and 50% effluent concentrations reached 100% mortality within 24 hrs of the initiation of the experiment. The concentration-response curve shows that at 20% effluent concentration all the organisms had died after 72 hrs, and 50% mortality was reached at 10% effluent concentration after 72 hrs (Figure 4.10). The calculated Probit LC50 was 11% with narrow 95% confidence limits ranging between 9 and 13% (Table 4.4). The low chi-square heterogeneity indicated a good fit of mortality data. The TSK method also indicated the suitability of the model, with the LC50 at 9% effluent concentration.

Experiment 4 General Textile Effluent (11-05-1998)

Description: *GTE Exp 4*

Exposure of baetids to GTE for 96 hrs was conducted at a range of effluent concentrations. Acclimation mortality was zero percent. *B. harrisoni* was the dominating species at 72% (Table 4.17). Responses are shown in Figures 4.11 and 4.12. The full chemical analysis is presented in Table 4.12.

Water quality

Table 4.7 presents ranges of physico-chemical variables monitored daily during Experiment 4.

TABLE 4.7
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 4

Para-meter (mg/l)	% Effluent concentration									
	0	**1**	**3**	**5**	**10**	**15**	**20**	**25**	**30**	**50**
EC (mS/m)	10.1-10.7	11.9-12.5	16.2-16.9	20.0-20.8	29.4-30.5	39.1-40.9	49.5-51.3	60.8-61.1	69.8-70.9	110.6-110.8
pH	7.1-7.4	7.2-7.5	7.7-7.8	7.9-8.9	8.0-9.6	8.1-9.7	8.3-10.1	9.4-10.3	9.5-10.4	9.8-10.7
Temp. (°C)	13.5-16.0	13.0-15.5	13-15.2	13.0-16.0	13.0-15.8	14.5-15.8	15.0-16.0	15.0-16.0	15.0-16.0	15.0-16.0

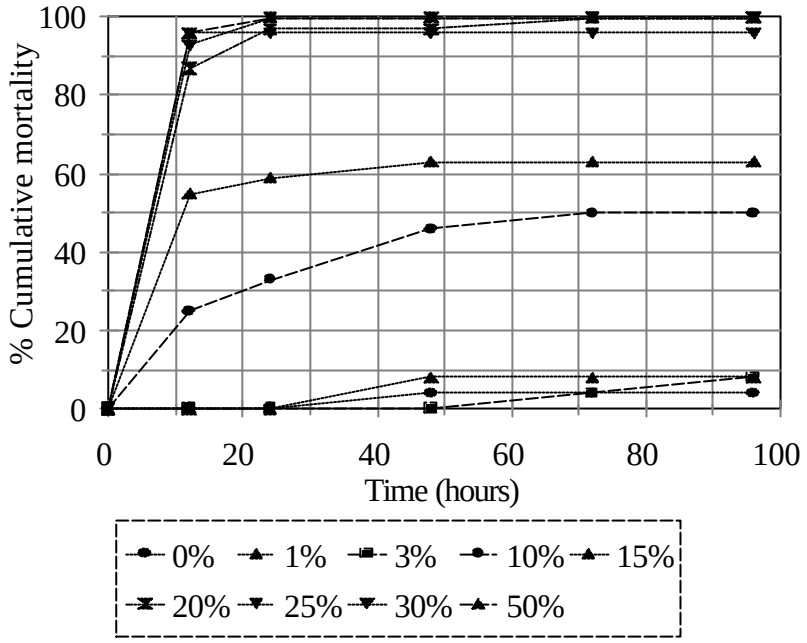

Figure 4.9 Experiment 3: The percentage cumulative mortality of baetids over 96 hrs, after exposure to a range of General Textile Effluent concentrations. The diluent was Buffalo River water.

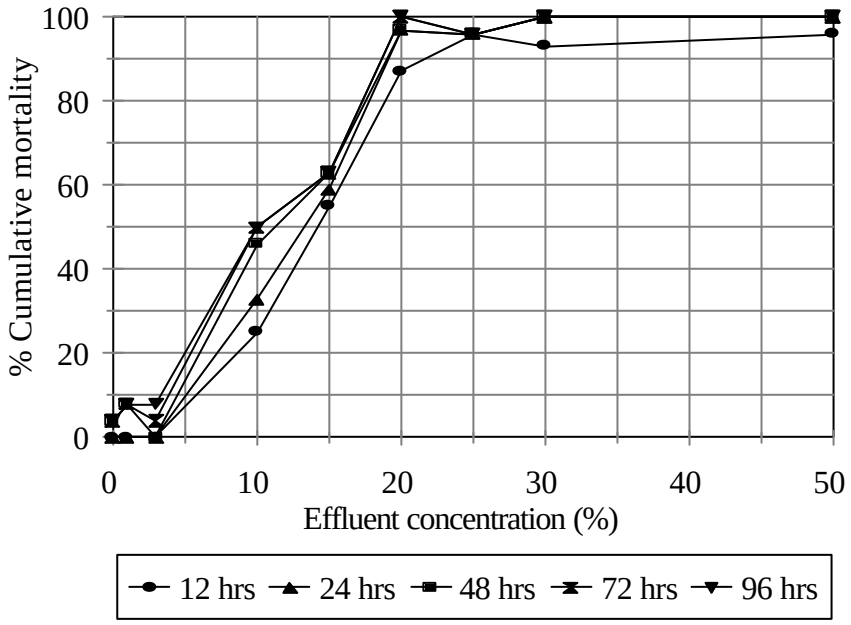

Figure 4. 10 Experiment 3: Concentration-response curve for baetids exposed to General Textile Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Buffalo River water.

The effluent was characterized by a blue-black colour. Its turbidity was also high. The effluent was alkaline, and the pH (Table 4.7) exceeded the recommended limit for the protection of aquatic environment (DWAF, 1999). The effluent EC was 261 mS/m at the start of the experiment (Table 4.12), and was higher than the recommended limit of 200 mS/m for irrigation with industrial effluent (DWAF, 1999), and was also higher than the EC for Experiments 1, 2 and 3. The sodium, potassium, chloride and TDS levels were all higher than all other GTE experiments (Table 4.12). This effluent was the most saline GTE sample tested in this study.

Toxicity results

After 96 hours, the control mortality was 8%. As low effluent concentration as 20% reached 100% mortality after 48 hrs (Figure 4.11). The concentration- response curve showed that at 7% effluent concentration, half of the test organisms had died within 48 hrs. The calculated Probit LC50 was 6% effluent concentration, with narrow 95% confidence limits, ranging between 4 and 8% concentration. The Trimmed Spearman-Karber results were similar to the Probit analysis (Table 4.3). Both methods showed this effluent to be the most toxic of the batches used.

Experiment 5 General Textile Effluent (15-05-1998)

Description: *GTE Exp5*

Responses of baetids to this batch of GTE are shown in Figures 4.13 and 4.14. Higher effluent concentrations were not used, due to the toxicity of GTE used for Experiment 4, and the effluent sample was taken within four days of Experiment 4. The responses were low, showing the low toxicity of the effluent. *A. parvum* was the dominating baetid species, making up 90% of the test population (Table 4.17).

Water quality

Table 4.8 presents the ranges of effluent concentrations of variables measured per channel during Experiment 5.

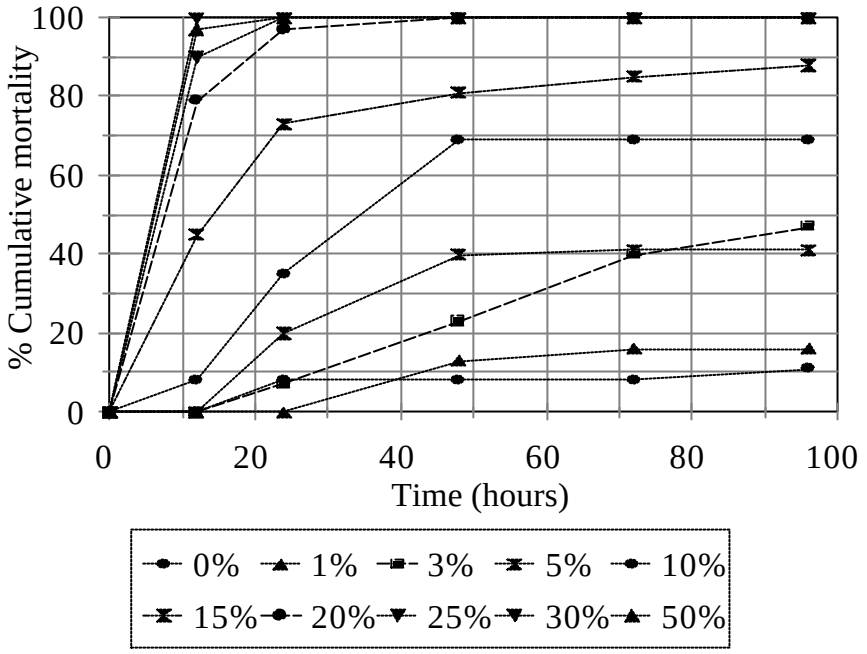

Figure 4.11 Experiment 4: The percentage cumulative mortality of baetids over 96 hrs, after exposure to a range of General Textile Effluent. The diluent was Buffalo River water.

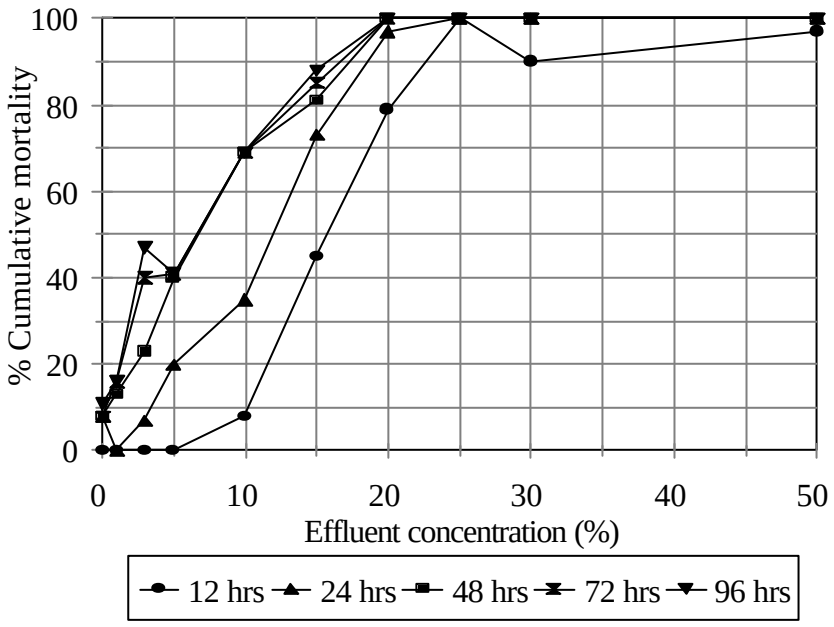

Figure 4. 12 Experiment 4: Concentration-response curve for baetids exposed to General Textile Effluent at a range of effluent concentration over various time periods (12-96 hrs). The diluent was Buffalo River water.

TABLE 4.8
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 5

Para-meter (mg/l)	% Effluent concentration					
	0	**1**	**3**	**5**	**10**	**15**
EC (mS/m)	23.7-27.2	25.2-29.0	28.0-31.7	30.3-35.8	28.2-42.0	45.2-51.0
pH	7.5-7.9	8.0-8.1	8.1-8.9	8.2-9.3	8.1-9.6	8.4-10.0
Temp. (°C)	13.0-18.0	12.8-18.0	12.8-18.0	12.8-18.0	12.8-15.0	13.0-18.2

The effluent was characterized by a blue-black colour. The observed turbidity was less than the previous GTE experiments. The EC for the effluent was 238.7 mS/m at the start of the experiment, and was comparable to the previous experiments. The pH of the effluent was alkaline. The channel temperatures fluctuated between 12.8 and 18.2°C, showing a variation of ± 5°C over 96 hrs.

Toxicity results

After 96 hrs, control mortality was 3%. After 96 hrs, very low responses were observed in the effluent concentrations used in the experiment. Both Probit and TSK analysis LC50 could not be calculated. The highest mortality was in 10% effluent concentration at 29%. When compared with the responses of the previous experiments, one would assume that this GTE would also show toxic responses. This could indicate the possibility of effluent variability.

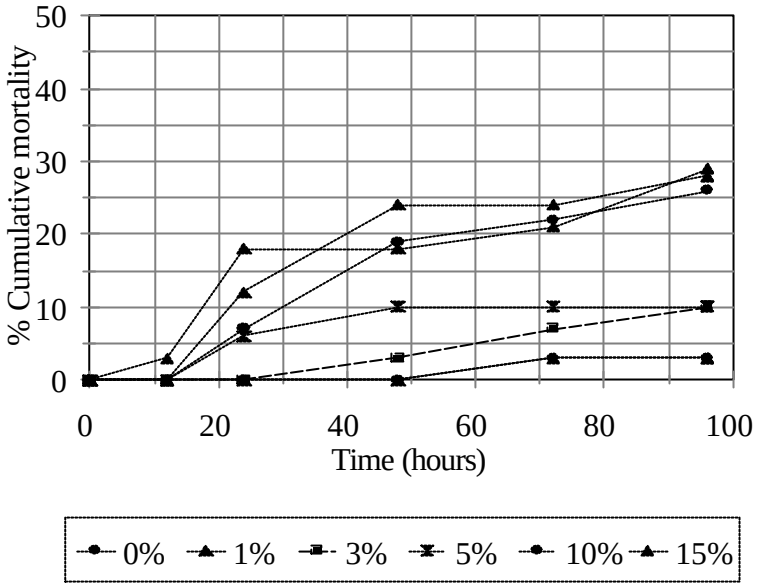

Figure 4.13 Experiment 5: The percentage cumulative mortality of baetids over 96 hrs, after exposure to General Textile Effluent, at a range of effluent concentrations. The diluent was Buffalo River water.

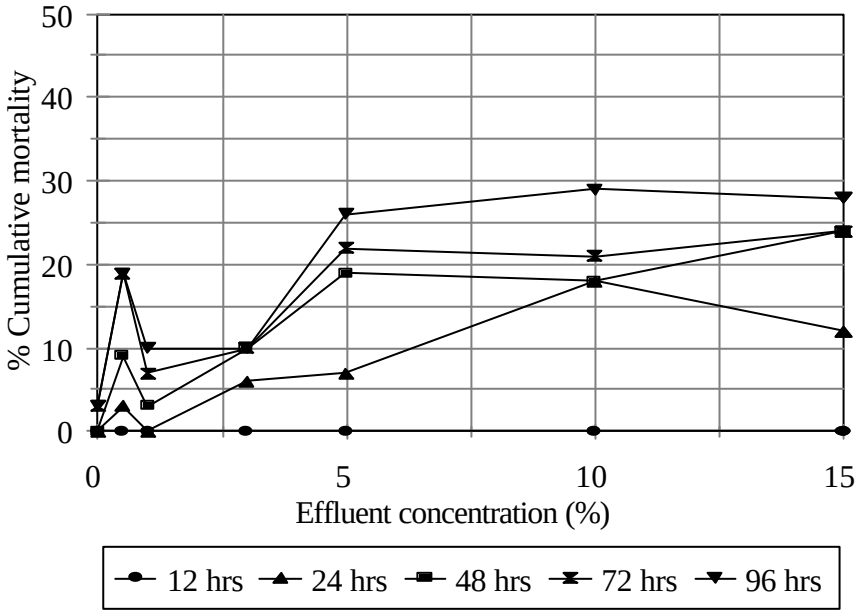

Figure 4. 14 Experiment 5: Concentration-response curve for beatids exposed General Textile Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Buffalo River water.

Experiment 6 Post Irrigation Textile Effluent (21-05-1998)

Description: *PITE Exp 6*

Baetids were exposed to a range of PITE concentrations for 96 hrs. A wide range of concentrations were used, so that a variety of responses could be obtained. Acclimation mortality was 3%. Responses are shown in Figures 4.15 and 4.16. The results show very low responses even at higher concentrations. The full physico-chemical analysis is shown in Table 4.13. *A. parvum* was the dominating test organism at 72% (Figure 4.17).

Water quality

Table 4.9 shows ranges of daily measured physico-chemical variables per channel, during 96 hrs toxicity testing.

TABLE 4.9
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 6

Para-meter (mg/l)	% Effluent concentration							
	0	**1**	**3**	**10**	**15**	**20**	**30**	**50**
EC (mS/m)	28.1-30.8	27.4-31.7	30.5-35.6	42.2-49.2	52.3-69.5	62.7-69.5	81.2-87.4	120.1-129.1
pH	7.3-8.1	7.6-8.3	8.0-8.4	8.3-8.5	8.4-8.7	8.5-8.7	8.5-8.8	8.5-8.9
Temp. (°C)	14.0-18.0	13.0-18.0	13.2-18.0	13.5-18.0	13.5-18.0	14.0-18.0	14.0-17.5	14.0-17.5

The effluent was characterized by a blue-black colour with less turbidity than GTE. The pH was within the General Standards (DWAF, 1991). The channel temperatures showed fluctuation of ± 4°C. The effluent EC was above the recommended limit for discharge into the aquatic environment (DWAF, 1996f). TDS, sodium and chloride levels were high, but were comparable to Experiments 4 and 5 (Tables 4.12 and 4.13), except for chloride levels. They were much higher than the other experiments, and were more than 20 fold that of the river water (Table 4.13).

Toxicity results

After 96 hrs, control mortality was 6%. Responses from this effluent were low compared to the responses of GTE. After 96 hrs, mortality at 25% effluent concentration was the highest at 29% (Figure 4.15). Responses did not follow any trend as effluent concentrations increased, instead, the concentration-response curve showed a fluctuation in responses (Figure 4.16). This is an indication that the test solution was not acutely toxic. The Probit and Trimmed Spearman-Karber LC50 values could therefore not be calculated (Table 4.3).

Experiment 7 Post Irrigation Textile Effluent (02-06-1998)

Description: *PITE Exp 7*

Baetids were exposed to PITE over 7 days (sub-chronic test), at a range of effluent concentrations (Table 4.1). Responses of test organisms are shown in Figures 4.17 and 4.18. Acclimation mortality was 6%. The full physico-chemical analysis results are presented in Section 4.5.4, Table 4.13. *A. parvum* was dominant at 67% (Table 4.17).

Water quality

Table 4.10 shows ranges of daily measured variables for individual channels during Experiment 7.

TABLE 4.10
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 7

Para-meter (mg/l)	% Effluent concentration										
	0	**1**	**3**	**5**	**10**	**20**	**30**	**50**	**60**	**75**	**100**
EC mS/m	32.7-39.4	34.0-40.3	35.5-45.3	41.1-48.6	50.0-58.2	69.0-73.8	86.2-97.5	120.0-137.2	138.0-150.7	162.4-181.6	202.4-221.6
pH	7.1-8.3	8.0-8.4	8.1-8.5	8.3-8.6	8.4-8.7	8.5-8.7	8.6-8.9	8.7-9.0	8.6-9.1	8.7-9.1	8.8-9.2
Temp (°C)	14.0-17.0	13.5-17.0	13.0-17.0	13.5-17.0	13.5-17.0	13.5-17.0	13.0-17.0	13.5-17.0	13.2-17.0	13.5-17.0	13.5-17.2

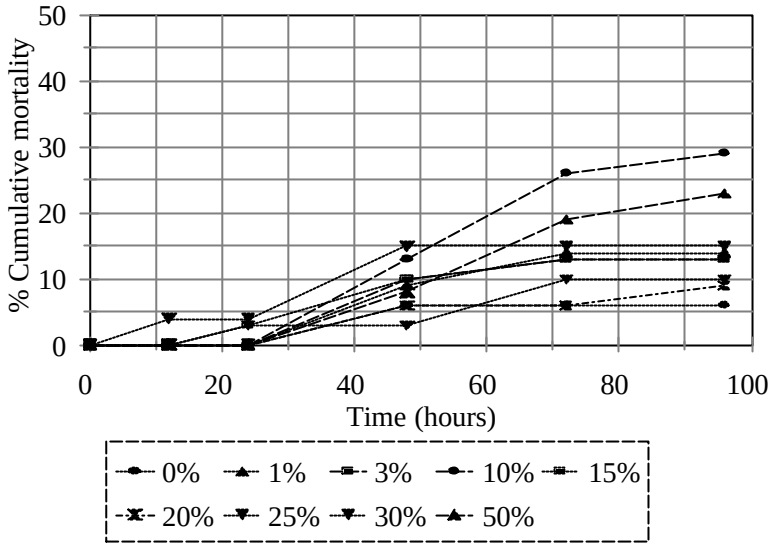

Figure 4.15 Experiment 6: The percentage cumulative mortality of baetids over 96 hrs, after exposure to Post-irrigation Textile Effluent, at a range of ffluent concentrations. The diluent was Buffalo River water.

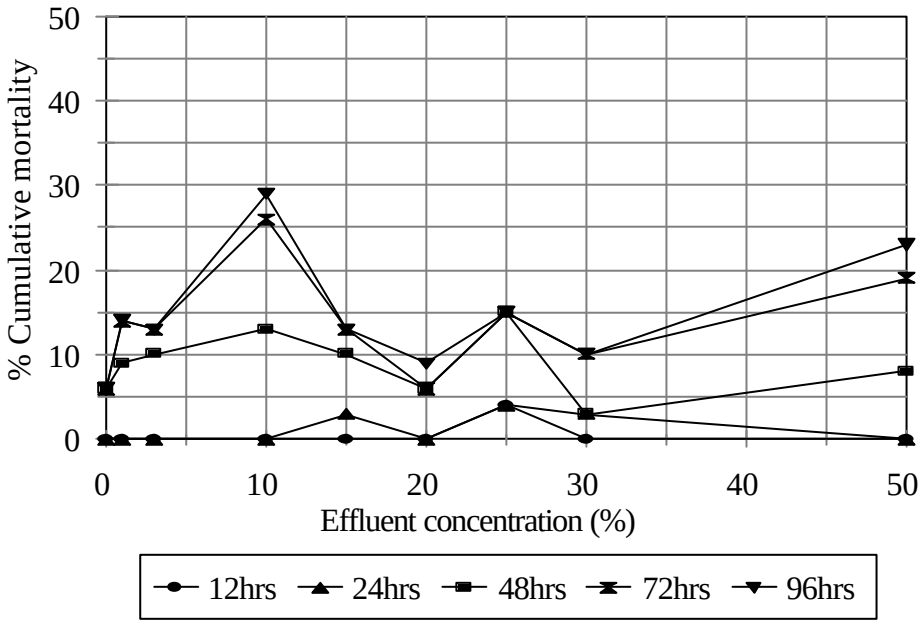

Figure 4.16 Experiment 6: Concentration-response curve for baetids exposed to Post Irrigation Textile Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Buffalo River water.

The effluent was characterized by a blue-black colour. It was less turbid than GTE. The EC was in the same range as that of GTE batches. The effluent was less alkaline with pH ranging between 7.1 and 9.2. The channel temperatures fluctuated within ± 4ºC (Table 4.10). TDS, sodium and chloride levels were high and comparable to the GTE effluent used during Experiment 6.

Toxicity results

Control mortality was zero after 96 hrs, but went up sharply to 10% within the next 72 hrs (i.e. Day 7). By the end of the experiment, 75% effluent concentration had the highest mortality at 30% (Figure 4.17). The concentration-response curve showed a fluctuation in responses as effluent concentrations increased (Figure 4.18). This seemed to indicate that the effluent was not very toxic over this experimental period.

Experiment 8 General Textile Effluent (11-11-1998)

Description: *GTE Exp 8*

Exposure of baetids to GTE for 96hrs was conducted at a range of concentrations (Table 4.1). Acclimation mortality was zero. Responses are shown in Figures 4.19 and 4.20. The results showed very low responses at low concentrations. In this experiment, *B. harrisoni* was the dominant species at 95% (Table 4.17).

Water quality

Table 4.11 presents daily measured variables per individual channel during Experiment 8.

TABLE 4.11
RANGES OF DAILY MEASUREMENTS PER CHANNEL (EFFLUENT CONCENTRATION IN %) DURING EXPERIMENT 8. * INDICATES THAT ONLY ONE READING TAKEN.

Parameter (mg/l)	% Effluent concentration									
	0	1	3	5	10	15	20	30	50	100
EC (mS/m)	23.8-25.5	24.9-27.9	28.0-31.7	31.5-34.8	39.3-43.6	48.1-54.1	54.5-61.3	70.8-77.8	104.3-119.8	232*
Temp. (°C)	19.0-21.0	18.8-20.5	19.0-20.5	19.0-21.0	19.0-20.5	19.0-20.5	19.0-20.5	19.0-21.0	18.8-21.0	20.5*

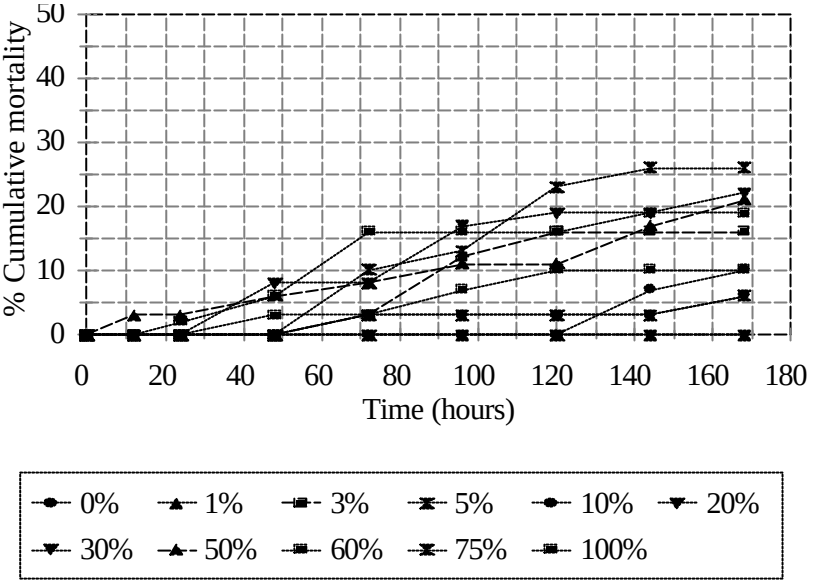

Figure 4.17 Experiment 7: The percentage cumulative mortality of baetids over 168 hrs, after exposure to Post-irrigation Textile Effluent, at a range of effluent concentrations. The diluent was Buffalo River water.

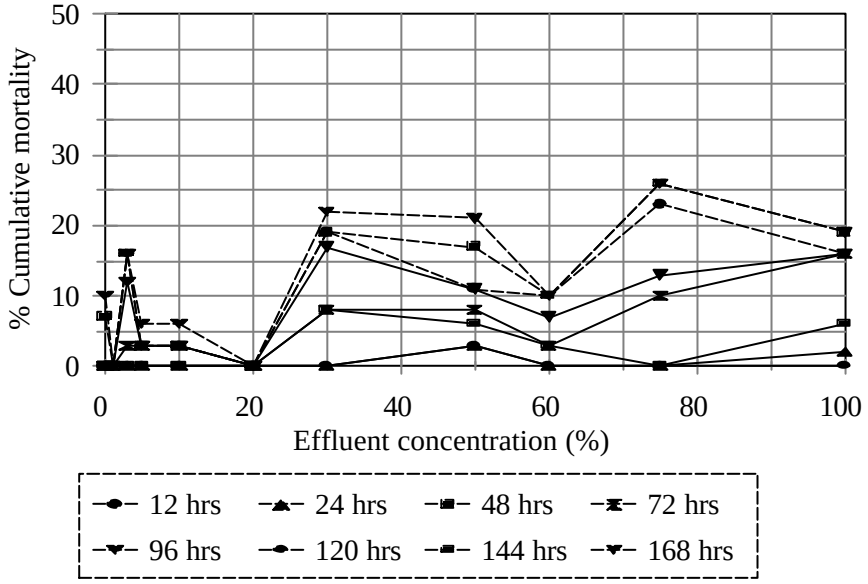

Figure 4.18 Experiment 7: Concentration-response curve for baetids exposed to Post Irrigation Textile Effluent at a range of effluent concentrations over various time periods (12-168 hrs). The diluent was Buffalo River water.

Effluent was characterized by a blue-black colour. Turbidity was high. EC was comparable to other effluent samples. The channel temperatures fluctuated between 18.8 and 21.0°C (Table 4.11), a variation of ± 2°C. The pH was not monitored due to a faulty pH meter.

Toxicity results

Control mortality was 8%, and mortalities for 1, 3 and 5% effluent concentrations were also below 10%. All the organisms in 30 and 50% effluent concentrations had died within 24 hrs. Once the 20% effluent concentration reached 60% mortality after 24 hrs, the graph levelled off until the end of the experiment (Figure 4.19), indicating that a toxicant threshold had been reached. The Probit method LC50 was calculated at 15% effluent concentration with a narrow 95% confidence limits, ranging from 13.0 to 17.0% effluent concentration. The TSK analysis LC50 value was similar to that of the Probit method (Table 4.3).

4.5.4 Analysis of water quality data

Whole effluent was analysed at the beginning and end of each experiment; results showed that various batches of effluents were chemically different from each other.

Tables 4.12 and 4.13 provide the individual experimental physico-chemical data analysis for GTE and PITE respectively**,** and show the chemical profile at the start (Day 0) and end (Day 4) of each experiment**.** Experiments 1 and 8 data are not available as analysis was incomplete due to insufficient sample.

The GTE was characterized by high salinity, with sodium and sulphate being major contributors. In comparison with the receiving Buffalo River water, GTE had a much higher conductivity (183-247mS/m), total alkalinity and nutrient concentrations. Sodium, chloride and potassium ions were all considerably elevated in the GTE.

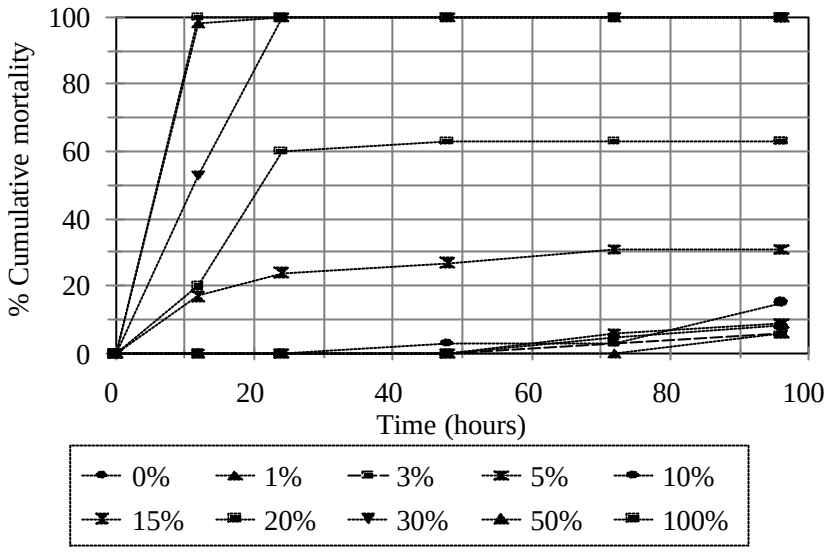

Figure 4.19 Experiment 8: The percentage cumulative mortality of baetids over 96 hrs, after exposure to General Textile Effluent, at a range of effluent concentrations. The diluent was Buffalo River water.

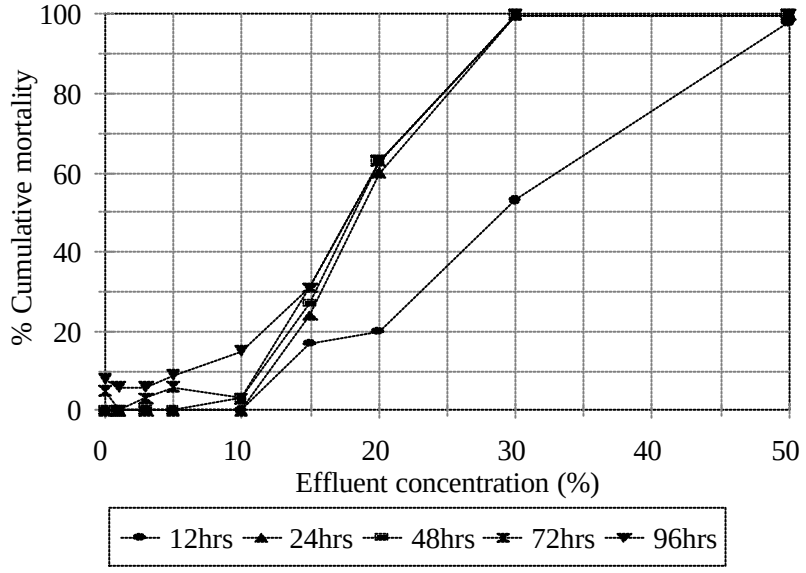

Figure 4. 20 Experiment 8: Concentration-response curve for beatids exposed to General Textile Effluent at a range of effluent concentrations over various time periods (12-96 hrs). The diluent was Buffalo River water.

The PITE is the run-off of irrigated GTE on grass pastures that collects in a holding dam, the Tailwater Dam. The EC, total alkalinity, trace metal ions and nutrients (particularly phosphates) are in the same range as GTE (Table 4.12). Chloride and calcium concentrations were elevated compared to the GTE (Table 4.12). There is a possibility that chloride and calcium ions are leaching from the soil in the irrigated area. Contaminated groundwater in the area (Bruinette *et al*., 1997), may also contribute to an increase of these salts.

TABLE 4.12
PHYSICO-CHEMICAL EFFLUENT CONSTITUENTS AND NUTRIENT CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING GENERAL TEXTILE MILL EFFLUENT TOXICITY EXPERIMENTS OVER SUMMER 1997, AUTUMN AND WINTER 1998. (EFFLUENT WAS ANALYZED BY IWQS). D0=START AND D4=END OF THE EXPERIMENT.

Parameter (mg/l)	Buffalo River water	Experiment 2		Experiment 3		Experiment 4		Experiment 5	
		D0	D4	D0	D4	D0	D4	D0	D4
EC (mS/m)	15.2	183	202	192	200	247	245	223	216
TDS	87.0	1669	1660	1607	1759	2173	2149	2031	1888
pH	7.7	8.2	8.4	8.5	8.4	8.6	8.6	8.5	8.3
TAL	35.0	624	629	586	666	917	900	838	745
SO_4^{2-}	7.0	273	261	260	273	227	228	259	261
Cl^-	13.0	114	114	141	141	144	134	121	139
Na^+	11.0	485	485	460	494	628	631	561	519
Mg^{2+}	4.0	4	3	2	3	2	2	4	3
K^+	1.1	19	18	17	19	32.9	33.3	29.3	27.1
Ca^{2+}	8.0	6	3	2	6	3	3	6	4
NH_4^+ –N	<0.04	2.9	3.3	6	4.6	12.6	13.9	20.8	19.4
NO_3^- +NO_2^- -N	<0.04	0.1	0.1	0.2	0.3	0.1	0.1	0.2	0.2
PO_4^{3-} – P	0.005	1	1.3	0.8	0.9	0.3	0.3	0.3	0.3
Cu-soluble	<0.005	DATA NOT AVAILABLE						0.4	0.3
Cr- soluble	<0.005							<0.04	<0.04
Zn- soluble	<0.005							1.6	1.7
Fe- soluble	<0.005							0.7	0.9

TABLE 4.13
PHYSICO-CHEMICAL EFFLUENT CONSTITUENTS AND NUTRIENT CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING POST-IRRIGATION TEXTILE EFFLUENT TOXICITY EXPERIMENTS OVER AUTUMN AND WINTER 1998. (EFFLUENT WAS ANALYZED BY IWQS). D0 = START AND D4 = END OF THE EXPERIMEN T.

Parameter (mg/l)	Buffalo River water	Experiment 6		Experiment 7	
		D0	D4	D0	D7
EC (mS/m)	15.2	254.0	262.0	223.0	256.0
TDS	87	2115	2147	2095	2081
pH	7.7	7.8	8.1	8.9	8.0
TAL	35.0	721	729	740	722
SO^{2-}_4	7.0	225	262	230	227
Cl^-	13.0	332	341	324	331
Na^+	11.0	616	590	606	609
Mg^{2+}	4.0	16.0	18.0	6.0	6.0
K^+	1.1	21.0	20.0	2.0	2.0
Ca^{2+}	8.0	18	19	17	18
NH_4^+ –N	<0.005	0.5	0.3	0.7	0.7
NO_3^- +NO_2^- -N	<0.005	0.3	0.2	0.1	0.2
PO_4^{3-}– P	0.03	1.90	1.80	1.90	1.90
Cu-soluble	<0.005	<0.05	0.40	<0.05	<0.05
Cr- soluble	<0.005	<0.05	<0.05	<0.05	<0.05
Zn- soluble	<0.005	<0.04	1.70	0.10	0.10
Fe- soluble	<0.005	0.8	0.9	0.8	1.8

4.5.5 Site-specific whole effluent guideline for textile effluent

There are no national numerical water quality standards for textile effluents in South Africa, and the need for these guidelines is growing due to the dumping of untreated effluent into the environment. The "polluter pays principle" is not being strictly enforced in South Africa. This principle was introduced to internalise the social cost of pollution rather than requiring a particular "end-of-pipe" treatment (Folkes, 1996). A method developed by Palmer and Scherman (in press), for describing toxicity data in hazard-based terms and relating these to the resource protection policy of DWAF (Palmer, 1999), was applied to each batch of textile effluent.

The AEV was calculated in each case according to the method of the DWAF (1996f), using LC1 values instead of a mean LC50 value as a single test species was used to generate toxicity data (Palmer and Scherman, in press) (Section 2.9.4). 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 (see Table 4.15). 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 (Table 4.14). 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 (Table 4.15A-D). The AEV was used as tolerance end-point to formulate the guidelines.

Table 4.14 provides a list of LC1, LC5, LC50 values and their 95%confidence limits, plus AEV values used in the ranking of tolerance end-points. Only Experiments 1, 3, 4 and 8 were included as both the Probit and TSK methods were not appropriate for Experiment 5, 6 and 7 data analysis.

TABLE 4.14
LC1, LC5 AND LC50 VALUES OF THE PROBIT METHOD FOR THE INDIVIDUAL GENERAL TEXTILE EFFLUENT EXPERIMENTS, THEIR 95% CONFIDENCE LIMITS AND AEV. (UCL=UPPER CONFIDENCE LIMIT, LCL=LOWER CONFIDENCE LIMIT)

Acute (96 hrs) test	LC1	LC1 95% LCL	LC1 95% UCL	LC5	LC5 95% LCL	LC5 95% UCL	LC50	LC50 95% LCL	LC50 95% UCL	AEV
Experiment 1	10.1	6.3	13.2	13.2	9	16.4	25	21.3	28	5.5
Experiment 3	5.1	2.6	6.9	6.5	3.9	8.2	11.4	9.3	12.9	2.5
Experiment 4	1.2	0.4	2.1	1.9	0.8	3	6	4.1	7.7	0.6
Experiment 8	6.3	3.7	8.3	8.2	5.8	10.1	15.6	13.5	17.4	3.1

Tables 4.15(A)-(D) show the ranking of toxicity test end-points, with a summarized specific hazard description, and associated river health Class (A-D and E/F), and a suggested textile whole effluent guideline as % effluent concentration. Classes A-D are ecologically sustainable and Classes E/F are degrading and degraded.

TABLE 4.15(A)
EXPERIMENT 1 (GTE): A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2), AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINES RANGES FOR TEXTILE EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1) AND HAZARD DESCRIPTIONS (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN (IN PRESS).

Tolerance test end-point	% effluent concen-tration	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 – 5
AEV LC1 lower 95% CL LC1	5.5 6.3 10.1	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	6 – 10
LC5 lower 95% CL LC1 upper 95% CL	9.0 13.2	Moderate hazard to intolerant biota: 95% probability of mortality between 1-5% after acute exposure	C	10 - 13
LC5	13.2	High hazard: best estimate of 5% mortality after acute exposure	D	13 - 16
LC5 upper 95% CL	16.4	Unacceptable hazard: 95% probability of at least 5% mortality after acute exposure	E/F	>16

TABLE 4.15(B)
EXPERIMENT 3 (GTE): A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2), AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR TEXTLIE EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1) AND HAZARD DESCRIPTIONS (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN (IN PRESS).

Tolerance test end-point	% effluent concen-tration	Summarized hazard description	River health class	Suggested % effluent concentration
Chronic test results not available	Unknown	Minimal hazard to intolerant biota- no acute responses	A	0 - 3
AEV LC1 lower 95% CL LC1	2.5 2.6 5.1	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	3 - 5
LC5 lower 95% CL LC1 upper 95% CL	3.9 6.9	Moderate hazard to intolerant biota: 95% probability of mortality between 1-5% after acute exposure	C	5 - 7
LC5	6.5	High hazard: best estimate of 5% mortality after acute exposure	D	7 - 8
LC5 upper 95% CL	8.2	Unacceptable hazard: 95% probability of at least 5% mortality after acute exposure	E/F	>8

TABLE 4.15(C)
EXPERIMENT 4 (GTE): A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2), AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR TEXTILE EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1) AND HAZARD DESCRIPTIONS (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN (IN PRESS).

Tolerance test end-point	% effluent concentration	Summarized hazard description	River health class	Suggested % effluent concentration
Chronic test results not available	unknown	Minimal hazard to intolerant biota- no acute responses	A	0 - 0.6
AEV LC1 lower 95% CL LC1	0.6 0.4 1.2	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	0.6 - 1.0
LC5 lower 95% CL LC1 upper 95% CL	0.8 2.1	Moderate hazard to intolerant biota: 95% probability of mortality between 1-5% after acute exposure	C	1 - 2
LC5	1.9	High hazard: best estimate of 5% mortality after acute exposure	D	2 – 3
LC5 upper 95% CL	3	Unacceptable hazard: 95% probability of at least 5% mortality after acute exposure	E/F	>3

TABLE 4.15(D)
EXPERIMENT 8 (GTE): A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2), AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR TEXTILE EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1) AND HAZARD DESCRIPTIONS (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN (IN PRESS).

Tolerance test end-point	% effluent concentration	Summarized hazard description	River health class	Suggested % effluent concentration
Chronic test results not available	Unknown	Minimal hazard to intolerant biota- no acute responses	A	0 – 3
AEV LC1 lower CL LC1	3.1 3.7 6.3	Low hazard to moderate biota: evidence of an acute response but 95% probability of less than 1% mortality after acute exposure	B	3 – 6
LC5 lower 95% CL LC1 upper 95% CL	5.8 8.3	Moderate hazard to intolerant biota: 95% probability of mortality between 1-5% after acute exposure	C	6 – 8
LC5	8.2	High hazard: best estimate of 5% mortality after acute exposure	D	8 – 10
LC5 upper 95% CL	10.1	Unacceptable hazard: 95% probability of at least 5% mortality after acute exposure	E/F	>10

Table 4.16 provides a list of experiments with associated river health classes and suggested guidelines for the textile effluents tested. From this data 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.

TABLE 4.16
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

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. Although PITE was found not to be acutely toxic, there is a potential for toxicity. The Management should therefore aim for zero effect to protect the environment.

4.5.6 Identification of test organisms

It was necessary to identify baetid mayfly nymphs used for toxicity tests, as different species may exhibit different sensitivities to the same toxicant. Using a test population consisting of one species is therefore recommended. Unfortunately, the Buffalo River baetid population did not consist of a single species, and it was necessary to speciate organisms upon the completion of the experiments. Table 4.17 presents a comparison of baetid population frequencies per experiment, i.e. during the summer of 1997 and 1998, and autumn and winter of 1998. During November 1997 and 1998, *B. harrisoni* was in the majority at 95% and *A. parvum at* 5%. *B. harrisoni* appeared to be a dominant species during summer months. This is possibly due to the fact that it is a more tolerant species, therefore less sensitive (Chutter, 1994), and can appeared to be

TABLE 4.17
COMPARISON OF BAETID POPULATION PERCENTAGE (%) FREQUENCY DURING THE TEXTILE ACUTE AND SUB-CHRONIC TOXICITY TES TING 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

a dominant species during summer months. This is possibly due to the fact that it is a more tolerant species, therefore less sensitive (Chutter, 1994), and 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.

4.6 DISCUSSION

4.6.1 Introduction

The discharge of untreated and partially treated effluent into the aquatic environment has frequently impaired the effective use of freshwater. Pollution has long-lasting effects, and may also have social impacts, as downstream users become affected by poor quality of water. This study used WET testing to provide a preliminary indication of potential effects of textile effluent on indigenous riverine invertebrates. This study, together with a kraft mill study (Chapter 3), can be seen as a first step in developing WET methods using indigenous riverine invertebrates. It also explores the link between the use of standard taxa and indigenous riverine invertebrates for toxicity testing.

The main finding of this study was that the textile effluent is variable and at times acutely toxic. The GTE was variable and more toxic than the PITE. The PITE collects into a holding dam, and settling of material takes place. This could have contributed to its stability. To achieve the aims of this study, baetid mayfly nymphs were exposed to a range of textile effluent concentrations for 96 hours, and one test was conducted for 7 days. The experiments were conducted in artificial stream systems (channels) using complex textile effluent as a toxicant, and river water as a diluent and a control. The results of this study were used to derive hazard assessment guideline for GTE, which could be used by the mill's management decision-makers about the treatment of effluent. This study was prompted primarily by the risk of textile effluents to the aquatic environment.

4.6.2 Textile effluents

The chemical source of textile effluent toxicity has been discussed at length in Section 4.1.2. The effluent was coloured as dyes and surfactants are passed onto the effluent during the manufacturing process. The combination of strong colour and high TDS levels lead to effluent turbidity in the experimental channels and it was difficult to see test organisms at high effluent concentrations. This could have also interfered with organism feeding. The GTE was more turbid than PITE; suggesting that turbidity

could have contributed to the death of organisms by clogging the gills. Effluents from the dyeing process are also toxic, and as most dyes are not biodegradable, they are not effectively removed during biological treatment.

Wool scouring is the major source of pollution in the textile industry (Correia *et al*., 1994), and therefore could have rendered the GTE toxic, as some scouring effluent is passed on to the next step of the manufacturing process. This textile mill uses hypochlorite for bleaching, and residuals and by-products would affect aquatic organisms (Nicolaou and Hadjivassilis, 1992).

In this study, the GTEs were generally found to be 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. Treated textile effluent has been found not to be toxic (Altinbas *et al*., 1995; Davies and Cottingham, 1994; Nicolaou and Hadjivassilis, 1992). The PITE was not acutely toxic although this effluent does not go through any form of biological treatment. 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 (Table 4.13). It has also been shown that Ca^{2+} has an ameliorative effect on toxicity (Palmer and Scherman, in press). There is also a possibility that non-toxicity is due to the fact that the effluent goes through a 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.

Effluent salinity

Information on the effects of salinity on riverine indigenous invertebrates (site-specific testing) appears to be scarce. The Centre for Aquatic Toxicology, at the Institute for Water Research, Grahamstown, initiated toxicity testing using indigenous riverine mayfly larvae, and selected salinity as the first water quality variable under

investigation. In the present study, textile effluent was used as a complex, saline whole effluent. Results showed that toxicity was not due only to increasing EC levels, as GTE and PITE exhibited similar Na^{+}, SO_4^{2-} and TDS levels, which contribute to salinity, and yet PITE was not acutely toxic. Calcium levels were however higher in the PITE (17 – 19 mg/l) than in the GTE (2 – 6 mg/l). The ameliorative effect of Ca^{2+} on salinity toxicity (Palmer and Scherman, in press), may have contributed to the reduced toxicity of the PITE. It is possible that the effluent salinity was not so high as to lethally affect organisms.

Generally, the salinity of the Buffalo River in the upper reaches is low (DWAF, unpubl. data), but discharges of treated and untreated sewage effluents and industrial effluents, such as tannery and textile effluents, have altered its salinity in the middle reaches. Salinity gradually increases as the river passes the industrial area, and decreases again to less than 50 mS/m, as the water leaves the Laing Dam. This indicates that self-cleansing takes place, and the dam acts as a settling reservoir. At Mc Tyre Bridge, a DWAF sampling point below the discharge point of the Mlakalaka stream, the salinity was higher by 2 folds than above the discharge point (DWAF, unpubl. data). The discharged textile effluent contributes to this increase in salinity. Salinity of textile effluents used in this study was 15 to 20 times that of the unimpacted Buffalo River water (at the sampling site), and has to some extent contributed to the increased salinity of the river. This becomes more evident during low flows, as the salinity of the Buffalo River middle reaches increase sharply (DWAF, unpubl. data). According to the present license conditions, the textile mill is allowed to discharge effluent with EC not greater than 250 mS/m. The new National Water Act (No 36 of 1998) has decreased the allowable EC to the maximum of 200 mS/m for irrigation (DWAF, 1999b).

Colour

Historically, textile effluents have a major environmental impact on rivers in industrial areas. As a result, the National Rivers Authority in the United Kingdom has set standards to protect key rivers affected by textile effluents, and has set target dates for compliance with the standards (ENDS Report, 1993). This has put pressure

on textile industries and Sewage Treatment Works. The Sewage Treatment Works were forced to reduce the levels of colour permitted in their trade effluent standards in order to comply. Colour is one of the most pressing problems facing the textile industry, and has the highest public profile. Up to 50% of the initial dye load will be found in the effluent (ENDS Report, 1993), giving rise to highly coloured effluent, which is difficult to treat.

The textile mill in this study is discharging coloured effluent into the receiving water via the Mlakalaka stream, as there are no strict regulations prohibiting the discharge. The strong colour of the textile effluent caused some change in the river water below the point of discharge. The colour change can cause a considerable disturbance to the ecological system of the receiving water.

4.6.3 Effluent, organism and experimental variability

Effluent variability arises both from the diversity in the types of industrial processes used, and the large range of chemicals and materials involved in the production of fabric. The composition of effluent is therefore influenced by a multitude of factors, i.e. the type of raw material, the textile process and its efficiency, and the effluent treatment. These factors result in textile effluent containing a complex variety of substances that have different effects on the aquatic environment. The combining of effluent streams from individual operation also result in large diurnal variations in effluent chemical composition, hence different organism responses from different batches. Variability in organism responses could also be due to genetic differences and individual test organism sensitivity. WET testing, as an experimental procedure, will also contribute some level of variability. It is important to know the acceptable levels of variability for compliance. CAT-IWR is presently compiling a document identifying and assessing sources of variability in toxicity testing using indigenuos invertebrates; and attempting to determine acceptable levels of variability for compliance and other applications of the method.

Although GTE samples for this study were collected from the same mill, utilizing the same manufacturing process, effluent chemical profile and subsequent organism responses were different.

Effluent variability

The complex composition and the continuos changing nature of the effluent (Rand, 1995), make minimizing the variability between organism responses over time difficult. Effluents can be highly variable over time, making toxic evaluations difficult (Warren-Hicks and Parkhurst, 1992; Dorn, 1996). There are currently no established criteria stipulating the acceptable levels of effluent variability in a WET test (Parkhurst and Mount, 1991), due to the inherent variability in effluent composition experienced over time. Average intra-laboratory coefficients of variation for acute WET test have been recorded as 17%, with as much as 135% variability being recorded between the experiments (Parkhurst and Mount, 1991). Such high variability between experiments can be expected if the composition of the effluent is highly variable. Understanding the effluent's variability may be more important than evaluating one toxic result as a significant event (Roux, 1994, Grothe *et al*., 1996).

In this study, selected physico-chemical constituents were used to compare the composition of different effluent batches (Tables 4.12 and 4.13). Chemical analysis of individual effluent showed no significant changes between the initial and the stored effluent over a four-day period. This indicates little variability within the effluent sample, which shows stability during storage. There was however, variability between the different effluent batches. This effluent was more variable, and produced more variable toxicity results than the kraft effluent used in Chapter 3. This effluent results show that it would not be possible to set the criteria or numerical value for effluents such as textile, as effluent produced differ all times.

Test organism variability

Riverine organisms from "unpolluted" water were used as test organisms, as suggested in APHA (1992). However, the organisms genetic structure was unknown, thereby introducing the variability associated with using a wild population of

organisms such as baetids. The use of such populations does incorporate natural variability, and therefore a measure of environmental realism that is not present in toxicity testing using standard laboratory organisms (Rosenberg and Resh, 1993). However, the test organisms were collected from one sampling site, thereby attempting to reduce a potential source of variability. The baetid population used for this study comprised a species complex; different species may respond differently to contaminant exposure (US EPA, 2000). As shown in Table 4.17, it is not possible to collect the same test species in the field, but an effort was made to try and select similar-looking organisms during sorting. Occasionally, it was necessary to use smaller-sized organisms due to low abundance of larger organisms, and this could have contributed to variability. The differences in species tolerance within a complex species may have contributed to variability.

Experimental variability

The channel temperatures fluctuated by more than ±3°C. A fluctuation of ±3°C over 24 hrs is considered acceptable under semi-controlled conditions (DWAF, 2000). These temperature fluctuations were due to sudden changes in laboratory temperature, which were influenced by the outside ambient temperatures. Although the recommended temperature fluctuation rate was exceeded, it did not appear to detrimentally affect test orgainsms, as control mortalities were below the 10% limit of DWAF (2000). The temperatures were still within the range that the organisms were naturally exposed to in the field, and suggests that test organisms are tolerant of a relatively wide temperature range. The river water temperatures fluctuate during the day, ranging from 13-18°C. Generally, if temperatures are allowed to fluctuate above the 18-20°C range, the life-cycle of mayflies becomes rapid, and as a result, emergence takes place (DWAF, 2000). In this study, there seems to be little correlation between poor temperature control and toxicity results (Experiments 2 and 3; Table 4.3 and Section 4.5.3). The use of a site-specific laboratory e.g. the Zwelitsha laboratory) for toxicity testing, reduces the control over physical parameters such as laboratory temperature. The effluent pH can influence the bioavailability of some metals (e.g. Cu and Zn), and therefore contribute to variability (US EPA, 2000). Strict control over abiotic factors must be exercised to reduce variability. Experimental

variability may be affected by dilution water quality, hence the use of river water as diluent in this study. In this study, the baetids percentage frequency data (Table 4.17) suggests that *B. harrisoni* is less sensitive than *A. parvum*.

Tolerance data

From the data tables and figures, it is apparent that test responses to each effluent batch was different. Out of eight different effluent samples, only four GTE showed acute toxic responses over 96 hrs. The effects varied in different batches, some showing immediate effects, while others showed delayed acute responses. Individual organisms of the same population could have responded differently from each other; effluent variability could also have contributed to different responses. Baetids showed less tolerance to GTE than PITE, indicating that PITE is more stable. The ameliorative effect of Ca^{2+} may also have contributed to the reduced toxicity of PITE.

4.6.4 Application of WET testing in South African water quality management

This study aimed to investigate the application of WET testing to complex textile effluents using indigenous riverine organisms as test organisms, to assess the potential effects of textile effluents on aquatic biota, and to use WET testing results in the development of hazard-based guidelines for textile effluent disposal. A hazard-based approach provides a consistent basis for deciding on the acceptability of impacts, while allowing natural site-specific differences to be taken into account (DWAF, 1999a). This study, together with the kraft mill effluent study (Chapter 3), must be seen as a first step in developing WET testing methods using selected indigenous riverine invertebrates. The data from this study may therefore be useful for setting whole effluent criteria for the protection of aquatic ecosystems.

In this study, WET testing was used to provide a preliminary indication of the potential effects of textile effluents on selected indigenous riverine invertebrates. Tolerance data were applied in a hazard-based manner to link the increasing risk of textile effluent impact to the river classification system and the determination of the ecological Reserve. The results gave a strong indication that GTE is acutely toxic, and

therefore should not enter the aquatic environment. The maximum concern of textile effluent which should enter a class C river, according to this preliminary study, should be 13% effluent concentration (Table 4.16). If a conservative approach is followed, the concern should be reduced to 1 – 2% effluent concentration. It is recognised that this was a very limited and preliminary study, but this "end-of-pipe" approach, utilizing toxicity data, follows the approach used in the DWAF draft guidelines for the assessment of, and authorization for, the discharge of complex waste to water resources. This document is currently being prepared by Palmer and Jooste (conf. draft). This study also clearly demonstrates the toxicity of textile effluents, and the highly variable nature of these effluents. These factors further strenghten the recommendation for the conservative management approach of these effluents.

The PITE was not acutely toxic, but probably chronically toxic. Few factors have probably contributed to less toxicity e.g. stabilization period in the Tailwater Dam; some of the substances in GTE were trapped in the soil during irrigation; and the ameliorative effect of Ca^{2+}.

The physico-chemical profile seemed to offer little information on the potential toxicity of the effluents, except for salinity effects. Chemical profiles also cannot predict the interactions between effluent components, and the effects of these interactions on effluent toxicity (Cairns *et al*., 1990). Differences in responses related to test organism and experimental variability could be monitored by the use of a reference toxicant. More work needs to be done to be able to establish what level of variability would be acceptable in compliance determination.

How indigenous test organisms could be used in a regulatory framework

The use of indigenous riverine invertebrates, fish and macrophytes for toxicity testing is ideal, as they reflect actual receiving water impacts. It is not known how the standard test organism and the international data compare to the responses of local species, therefore it is difficult to evaluate the real level of protection indigenous organisms would offer as test species. Since the use of indigenous invertebrates increases the reliability of data and environment realism, it could be useful in setting

site-specific guidelines. The use of indigenous organisms, representing a wild population, generates responses that include natural variability.

In this study, baetids showed sensitivity to GTE. 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. Since it is not always practical to collect enough numbers of organisms for toxicity testing, indigenous riverine invertebrates could be used for auditing rather than routine monitoring.

To conclude, the results of this study indicated that the GTE should not enter the river, as it will have detrimental impacts on the aquatic environment. The GTE proved to be variable and acutely toxic; PITE was more stable and not acutely toxic. Baetid responses demonstarted their sensitivity to textile effluents. Using baetids as test organism for routine testing will not be practical, as their availability depends on natural factors such as flow. The use of a baetid species complex is also not recommended, as different baetid species show a range of responses to toxicants. The management of the mill should therefore consider focussing on an Environmental Risk Assessment approach, and use it as a tool for its environmental decision-making. Although preliminary, hazard-based guidelines, which relate textile effluent toxicity to river health, have been provided as a starting-point. These data would allow the factory managers to relate specific effluent concentrations to the likely risk to in-stream biota.

4.6.5 Recommendations

Recommendations for future research

The work done in this study provided useful information and data, but was preliminary. There is a need to have more data on how indigenous riverine invertebrates respond to potential pollutants, both single-substance and complex effluents. Groundwater testing is necessary, to determine whether irrigating with the

textile effluent impacts on groundwater. This is particularly relevant as the kraft effluent preliminary study (Section 3.5.6) showed irrigation impacting on groundwater. Although the results showed baetids to be sensitive to textile effluent, it cannot be assumed that guidelines protecting these organisms would protect all other organisms. *Daphnia* toxicity testing, and testing using organisms such as fish and algae, should be undertaken, and sensitivities compared with that of baetids. There is also a need to investigate chronic and long-term responses, especially for PITE, which did not show acute toxicity. In-stream biomonitoring studies should also be undertaken and results linked to toxicity testing to increase chances of protection of the aquatic biota.

Recommendations for textile management

The textile mill management should consider treating their effluent before discharging or irrigating, as it is probable that textile effluent will reach the receiving water under the present scenario. This will also help to reduce the amount of effluent to be disposed, as some of the effluent could be re-used. A hazard-based site-specific approach is also recommended, with the mill being conservative, i.e. aiming for zero impact. This is particulary important due to the toxicity of this effluent (see Table 4.16). The quantity or flow and quality of river water will also determine the impact of effects of the effluents (Smith and Sprague, 1992). To be able to monitor the impact of the discharged effluent, biomonitoring at, above, and below the point of discharge should be undertaken. Toxicity testing should be conducted using *Daphnia* for routine monitoring, and indigenous invertebrates such as baetids, for auditing purposes.

CHAPTER 5

CONCLUDING DISCUSSION

5.1 INTRODUCTION

Water is scarce in South Africa, and therefore needs to be conserved and protected, so as to ensure sustainability (DWAF, 1997a). To be able to protect water resources, wastewater discharged into the aquatic environment must not lead to irreversible or unacceptable impacts. However, South African researchers only recently produced a standardized test protocol for the use of indigenous organisms in toxicity tests (DWAF, 2000). This study was the first in South Africa to formulate hazard-based guidelines for complex effluents using WET data, and therefore constitutes a potential method to monitor and manage water resource use at sustainable levels. In this concluding discussion, the limitations of this approach; problems encountered during the study, and the application of the results to management are considered.

5.2 LIMITATIONS, ADVANTAGES AND PROBLEMS EXPERIENCED DURING THE STUDY

a) *Kraft mill study*

- Effluent composition is usually variable, and effluents which are discharged after an industrial process without storage, as is the case here, are particularly variable (Grothe *et al*., 1996). In this study, only seven acute (96 hr) and one sub-chronic (7 day) tests were conducted, unfortunately not generating information on an annual cycle of variation (Slabbert *et al*., 1998a). The scope of this study was therefore not sufficiently frequent or long-term to quantify effluent variability.
- Groundwater will be influenced by factors other than the effluent, therefore results from this study will give at best, a "snapshot" of possible effects.
- Test organisms and river water used for the study were from the Sabie River (Sabie-Sand River system), which is a different system from the receiving

water (the Elands River is part of the Crocodile River system). This is not an ideal situation, but the Sabie River had a known, well-established test population of the mayfly *T. tinctus*, whose responses to elevated salts were already known (Palmer and Scherman, in press). Since the test effluent was variable, and the responses of indigenous organisms are variable (Palmer and Scherman, in press), it seemed best to select a previously studied test population. *T. tinctus* does occur in the Elands River, so the results could be extrapolated with reasonable confidence.

- The laboratory temperatures could not be adequately controlled to a constant temperature in the Skukuza site-specific laboratory. One of the air-conditioners became faulty during the experiment and tripped the electricity in the laboratory; resulting in a disruption of power to some artificial streams. This could have been responsible for the outliers identified during the statistical analysis.
- The space in the laboratory was a limiting factor in the number of streams used for the experiments, hence replication was not possible.
- The distance between the effluent collection site and the laboratory where experiments were conducted, was problematic.
- The first batch of effluent foamed profusely during the experiment, creating problems when running 50 – 100% effluent concentrations.
- Some batches of effluent had high levels of suspended solids, which caused clogging.

b) *Textile mill study*

- Test organisms were a mixed population, and could not be identified to species level before the experiment was initiated. The availability of organisms was seasonal and the numbers declined as the river flow and volume decreased. Although *B. harrisoni* is known to be a tolerant species (Chutter, 1994), a pattern could not be identified between species dominance and LC50 values.
- The selection of test organisms was based on their abundance in the Buffalo River, their suitability to the test systems, and their use in other studies (Williams, 1996).
- Baetids may not be the ideal organism for routine testing, as the study has shown that

their numbers are drastically reduced during low flow conditions, therefore there is no guarantee of availability all year round.

- The control of laboratory temperatures was not very successful (temperatures fluctuated between 13-21˚C), and was influenced by the outside temperatures. Once the outside temperatures dropped at night, the temperatures inside the laboratory would also drop. This did not seem to influence the results as control mortalities were always below 10%. It is possible that test organisms are acclimated to fluctuations in temperature as river water temperature also fluctuated to low temperatures.
- Effluent composition was complex and usually variable. (If the effluent is stored in a large holding dam (see Chapter 4) or lagoon, with a long retention time prior to discharge, then the effluent is likely to be relatively stable (Burton Jr. *et al*., 1996)).

WET testing is relatively new in South Africa (Slabbert *et al*., 1998a), 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, 1996f) 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 macro-invertebrates 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.

5.3 WET TESTING AND COMPLEX EFFLUENT MANAGEMENT

WET testing has the advantage of measuring the effects of complex effluents in a form that is bioavailable to organisms; it incorporates the effects of interactions of constituents (US EPA, 1992). It also has the following potential disadvantages (US EPA, 1992):

- It does not indicate how to treat effluent toxicity or variability;
- it provides no information about protecting human health;
- it does not indicate how long toxicity persists in the environment; and
- it does not take into account the changes in toxicity that can result from environmental changes.

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. *T. tinctus* 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 (Palmer and Scherman, in press), as Ca 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 recommends a 3% effluent concentration guideline for both general kraft effluent (based on mean test results, Table 3.19) and general textile effluent (based on mean test results, Table 4.16) for the protection of a Class A river. Ranges for other river

classes were also derived. Table 5.1 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 5.1 shows a list of the recommended hazard-based % effluent guidelines for general kraft and textile effluents.

TABLE 5.1
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 health 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 pulp and paper kraft 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* (Chapter 3) confirmed the findings documented in the literature that untreated pulp and paper kraft effluents are toxic. The effluent batches sampled in 1997 were more acutely toxic than effluents sampled in 1998. 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, 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. River water showed no toxicity, indicating the good quality of the receiving water. Observed responses were therefore due to the toxicant.

The percentage frequency of baetids used for textile effluent testing (Table 4.17), 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.

PITE toxicity results suggest that evaporation (during irrigation) and stabilisation (in the form of degradation and settling) in the holding dam may have reduced toxicity. The fact that PITE was less toxic than GTE does not mean that it does not impact on the environment. Long-term chronic exposure may show PITE to be toxic.

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 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 in aquatic environment should be prohibited.

To conclude, WET 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.

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APPENDIX A

Physico-chemical constituents of kraft effluent

TABLE A 1

EFFLUENT VARIABILITY: PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENT CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING GENERAL KRAFT EFFLUENT TOXICITY EXPERIMENTS OVER WINTER 1997. (EFFLUENT WAS ANALYZED BY IWQS). D0 = FIRST DAY; D4 = LAST DAY

Parameter (mg/l)	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
	D0	D4	D0	D4	D0	D4	D0	D4
EC (mS/m)	408.0	425.0	138.0	134.0	343.0	350.0	141.0	108.9
TDS	2286.0	2369.0	1034.0	995.0	3430.0	3184.0	1156.0	900.0
pH	8.3	8.7	7.2	7.4	10.3	10.1	8.1	7.5
TAL	15.0	<4.0	116.0	126.0	<4.0	<4.0	353.0	286.0
SO_4^{2-}	974.0	1086.0	517.0	485.0	637.0	622.0	314.0	236.0
Cl^-	45.0	43.0	49.0	46.0	29.0	35.0	50.0	38.0
Ca^{2+}	48.0	66.0	45.0	44.0	20.0	13.0	25.0	20.0
Mg^{2+}	33.0	53.0	7.0	7.0	21.0	13.0	3.0	3.0
Na^+	1115.0	1069.0	249.0	237.0	703.0	747.0	319.0	244.0
NH_4^+ - N	0.1	0.1	1.9	2.5	5.8	3.6	0.06	0.07
$NO_3^- + NO_2^-$ -N	<0.04	<0.04	0.3	0.08	<0.04	<0.04	<0.04	0.1
PO_4^{3-}-P	<0.005	<0.005	<0.005	0.06	645.8	559.8	0.04	<0.005
Fe- soluble	ND	0.9	1.0	0.8	1.3	1.3	0.2	ND
Zn-soluble	ND	0.8	0.09	0.3	0.3	0.6	0.5	ND
B-soluble	ND	1.1	0.4	0.3	0.3	0.8	0.8	ND
Mn-soluble	ND	2.4	2.2	1.9	<0.001	1.4	0.4	ND
Al-soluble	ND	4.3	1.3	0.9	2.2	3.1	0.5	ND

ND = not done

TABLE A 2

EFFLUENT VARIABILITY: PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENT CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING GENERAL KRAFT EFFLUENT TOXICITY EXPERIMENTS OVER WINTER 1998. (EFFLUENT WAS ANALYZED BY IWQS).

D0 = FIRST DAY; D4 = LAST DAY

Parameter (mg/l)	Experiment 5		Experiment 6	
	D0	D4	D0	D4
EC (mS/m)	110.0	ND	151.0	161.0
TDS	802.0	ND	1181.0	1252.0
pH	7.3	ND	6.9	7.9
TAL	114.0	ND	368.0	376.0
SO_4^{2-}	300.0	ND	259.0	302.0
Cl^-	60.0	ND	76.0	79.0
Ca^{2+}	25.0	ND	203.0	206.0
Mg^{2+}	7.0	ND	12.0	12.0
Na^+	144.0	ND	164.0	175.0
NH_4^+ - N	<0.04	ND	0.05	0.05
NO_3^- + NO_2^- -N	<0.04	ND	0.05	0.06
PO_4^{3-} P	<0.005	ND	<0.005	<0.005
Fe-soluble	0.9	ND	ND	0.9
Zn-soluble	<0.005	ND	ND	0.2
B-soluble	<0.005	ND	ND	0.3
Mn-soluble	1.8	ND	ND	3.3
Al-soluble	1.3	ND	ND	0.3

ND = not done

TABLE A 3
PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENT CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING GENERAL KRAFT EFFLUENT EXPERIMENT 5, 1998

Parameter (mg/l)	% effluent concentration						Control
	1	3	10	30	50	100	
EC (mS/m)	12.3	ND	23.7	32.7	55.7	110.0	15.3
TDS	106.0	ND	164.0	279.0	401.0	802.0	117.0
pH	7.9	ND	7.4	7.9	7.3	6.9	8.0
TAL	9.0	ND	31.0	95.0	159.0	259.0	7.0
SO_4^{2-}	<25.0	ND	<25.0	<25.0	29.0	60.0	<25.0
Cl^-	9.0	ND	13.0	19.0	25.0	25.0	10.0
Ca^{2+}	6.0	ND	6.0	7.0	7.0	7.0	7.0
Mg^{2+}	7.0	ND	19.0	48.0	77.0	144.0	7.0
Na^+	0.1	ND	<0.04	<0.04	<0.04	<0.04	<0.04
NH_4^+ - N	0.2	ND	<0.04	0.1	<0.04	<0.04	0.2
NO_3^- + NO_2^- -N	<0.005	ND	<0.005	0.1	<0.005	<0.005	0.02
PO_4^{3-}-P	0.1	0.1	0.2	0.2	0.5	0.9	<0.005
Fe-soluble	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Zn-soluble	<0.005	<0.005	<0.005	<0.005	<0.005	0.2	<0.005
B-soluble	<0.005	<0.005	<0.005	<0.005	0.15	0.3	<0.005
Mn-soluble	<0.001	<0.001	<0.001	0.2	0.4	3.3	<0.001
Al-soluble	0.1	<0.02	<0.02	<0.02	0.3	0.3	<0.02

ND = not done

TABLE A 4
PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENT CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING GENERAL KRAFT EFFLUENT EXPERIMENT 6, 1998

Parameter (mg/l)	% effluent concentration						Control
	1	3	10	30	50	100	
EC (mS/m)	16.3	18.7	31.3	54.1	81.3	158.5	14.4
TDS	127.5	151	240	481	744	1234.5	107
pH	7.7	7.7	7.8	7.6	7.5	7.7	7.9
TAL	9.5	15	31	91	165	291.5	7.0
SO_4^{2-}	<25	<25	<25	29	44	78.0	<25
Cl^-	12.0	16.5	29.0	70.0	110.0	205.2	10.0
Ca^{2+}	7.0	6.5	8.0	9.0	10.0	12.0	7.0
Mg^{2+}	8.0	10.5	21.0	54.0	91.0	172.2	7.0
Na^+	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
NH_4^+ - N	<0.04	<0.04	<0.04	<0.04	<0.04	0.1	0.1
$NO_3^- + NO_2^-$ -N	<0.005	0.02	<0.005	<0.005	<0.005	<0.005	<0.005
PO_4^{3-}-P	<0.005	0.1	0.1	0.3	0.5	0.9	5.15
Fe-soluble	<0.005	<0.005	<0.005	<0.005	0.05	0.2	<0.005
Zn-soluble	<0.005	<0.005	<0.005	0.1	0.2	0.3	<0.005
B-soluble	<0.001	0.1	<0.001	<0.001	1.3	3.3	<0.001
Mn-soluble	<0.005	0.1	<0.005	<0.005	<0.005	0.3	<0.005

ND = not done

TABLE A 5

PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENTS CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING IRRIGATION KRAFT EFFLUENT TOXICITY EXPERIMENT 7, 1998

Parameter (mg/l)	% effluent concentration							Control
	1	3	10	30	50	75	100	
EC (mS/m)	18.7	32.7	29.5	ND	150.0	234	252.2	15.3
TDS	133.0	209.0	190.0	ND	1122.0	1483	1904.0	117.0
pH	8.1	7.4	7.3	ND	7.5	6.9	6.5	8.0
TAL	14.0	240	27.0	ND	236.0	309.0	401.0	7..0
SO_4^{2-}	<25	42.0	36.0	ND	350.0	481.0	630.0	<25.0
Cl^-	11.0	14.0	11.0	ND	37.0	48.0	60.0	10.0
Ca^{2+}	7.0	8.0	7.0	ND	17.0	20.0	24.0	7.0
Mg^{2+}	12.0	37.0	32.0	ND	319.0	423.0	564.0	7.0
Na^+	<0.04	<0.04	<0.04	ND	0.1	0.1	0.1	<0.04
NH_4^+ - N	<0.04	<0.04	<0.04	ND	<0.04	0.04	<0.04	0.2
$NO_3^- + NO_2^-$ -N	0.02	<0.005	<0.005	ND	0.2	0.3	0.4	0.02
PO_4^{3-}-P	0.1	0.1	0.2	0.5	1.1	1.03	1.3	<0.005
Fe-soluble	<0.005	<0.005	<0.005	<0.005	<0.005	0.05	<0.005	<0.005
Zn-soluble	<0.005	<0.005	<0.005	0.1	0.1	0.1	0.3	<0.005
B-soluble	<0.001	<0.001	0.2	0.9	1.4	2.5	3.1	<0.001
Mn-soluble	<0.005	<0.001	0.1	0.5	0.7	1.1	1.6	<0.001

ND = not done

TABLE A 6

PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENTS CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING IRRIGATION KRAFT EFFLUENT TOXICITY EXPERIMENT 8, 1998

Parameter (mg/l)	% effluent concentration							Control
	1	3	10	30	50	75	100	
EC (mS/m)	18.7	25.5	29.5	ND	144.0	199.0	310.0	15.3
TDS	133.0	174.0	190.0	ND	859.0	1478.0	1940.0	117.0
pH	8.1	7.7	7.3	ND	7.5	6.9	6.7	8.0
TAL	14.0	32.0	27.0	ND	157.0	310.0	378.0	7.0
SO_4^{2-}	<25.0	28.0	36.0	ND	282.0	472.0	661.0	<25.0
Cl^-	11.0	12.0	11.0	ND	31.0	48.0	58.0	10.0
Ca^{2+}	7.0	7.0	7.0	ND	15.0	20.0	23.0	7.0
Mg^{2+}	12.0	23.0	32.0	ND	243.0	424.0	589.0	7.0
Na^+	<0.04	<0.04	<0.04	ND	0.1	0.1	0.1	<0.04
NH_4^+ - N	<0.04	<0.04	<0.04	ND	<0.04	<0.04	<0.04	0.2
$NO_3^- + NO_2^-$ -N	<0.005	<0.005	<0.005	ND	0.2	0.3	0.5	<0.005
PO_4^{3-}-P	0.1	0.1	0.1	0.6	0.7	0.9	0.1	<0.005
Fe-soluble	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
Zn-soluble	<0.005	<0.005	<0.005	0.1	<0.005	0.2	0.2	<0.005
B-soluble	0.1	<0.001	<0.001	0.9	1.3	2.2	3.0	<0.001
Mn-soluble	<0.005	<0.005	<0.005	0.4	0.6	1.3	1.6	<0.005

ND = not done

TABLE A 7

PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENTS CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING IRRIGATION KRAFT EFFLUENT TOXICITY EXPERIMENT 9, 1998

Parameter (mg/l)	% effluent concentration							Control
	1	3	10	30	50	75	100	
EC (mS/m)	17.9	ND	62.3	139.5	199.0	305.0	395.0	13.6
TDS	146.0	ND	222.0	849.0	1333.0	2099.0	2536.0	12.0
pH	8.0	ND	7.8	7.3	7.0	6.9	6.9	7.9
TAL	12.0	ND	65.0	150.0	242.5	4580	465.0	7.0
SO_4^{2-}	<25.0	ND	<25.0	270.5	445.5	742.0	897.0	<25.0
Cl^-	11.0	ND	17.0	26.5	37.5	54.0	61.0	10.0
Ca^{2+}	8.0	ND	7.0	17.5	24.5	37.5	37.0	7.0
Mg^{2+}	15.0	ND	12.0	229.5	377.0	615.5	776.0	7.0
Na^+	<0.04	ND	<0.04	0.3	0.6	1.0	1.4	<0.04
$NH_4 - N$	<0.04	ND	<0.04	0.1	0.1	0.1	0.2	<0.04
$NO_3 + NO_2 - N$	<0.005	ND	<0.005	0.0	0.0	0.0	0.1	0.1
$PO_4^{3-} - P$	<0.005	0.1	0.1	0.6	0.9	1.1	9.1	5.1
Fe-soluble	<0.005	<0.005	<0.005	<0.005	0.1	0.1	0.1	<0.005
Zn-soluble	<0.005	<0.005	<0.005	0.1	0.1	0.1	0.2	<0.005
B-soluble	<0.001	<0.001	<0.001	1.6	3.0	4.2	5.2	<0.001
Mn-soluble	<0.005	<0.005	<0.005	0.4	0.3	0.5	0.6	<0.005

ND = not done

TABLE A 8

PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENTS CONCENTRATIONS (EXPRESSED IN MG /L) MONITORED DURING IRRIGATION KR AFT EFFLUENT TOXICITY EXPERIMENT 10, 1998

Parameter (mg/l)	% effluent concentration							Control
	1	3	10	30	50	75	100	
EC (mS/m)	18.0	28.9	48.7	138.2	183.5	257.0	324.0	14.4
TDS	136.0	186	358.0	863.5	1359.5	1906	2447.0	107.0
pH	7.9	7.7	7.9	7.1	7.0	6.7	6.6	7.9
TAL	12.0	19.5	52.0	149.5	248.0	407.0	488	7.0
SO_4^{2-}	<25.0	34.5	98.0	279.5	458.5	660.5	852.0	<25.0
Cl^-	10.5	12.0	16.0	28.0	37.5	50	59	10.0
Ca^{2+}	7.5	8.5	11.0	17.0	25.5	33.0	39.0	7.0
Mg^{2+}	13.5	30.5	83.0	235	390.5	561.0	720.0	7.0
Na^+	<0.04	0.01	<0.04	0.3	0.6	0.9	1.3	<0.04
NH_4^+ - N	0.1	<0.04	0.1	0.1	0.1	0.1	0.2	<0.04
$NO_3^- + NO_2^-$ -N	0.1	<0.005	<0.005	<0.005	<0.005	0.1	0.1	0.1
PO_4^{3-} - P	<0.005	<0.005	0.1	0.2	0.7	1.6	1.2	5.15
Fe-soluble	<0.005	<0.005	<0.005	<0.005	0.05	0.1	0.1	<0.005
Zn-soluble	<0.005	<0.005	<0.005	<0.005	0.0	0.05	0.1	<0.005
B-solble	<0.001	<0.001	0.05	1.25	2.95	4.05	5.6	<0.001
Mn-soluble	<0.005	0.0	0.05	0.35	0.6	1.05	1.4	<0.005

ND = not done

APPENDIX B

Probit method and Trimmed Spearman-Karber analyses data

Probit method

Kraft effluent

Experiment 1 GKE

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
0.5000 33	0	0.000	0.000	0.000	
1.0000	36	0	0.000	0.000	0.000
2.0000	32	0	0.000	0.000	0.001
3.0000	35	0	0.000	0.000	0.004
4.0000	36	1	0.028	0.029	0.014
5.0000	32	1	0.031	0.031	0.029
10.0000	36	7	0.194	0.194	0.177
30.0000	31	18	0.581	0.581	0.729
50.0000	31	31	1.000	1.000	0.907

Chi - Square Heterogeneity = 7.429

Mu = 1.288
Sigma = 0.310

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	0.852	0.454	(-0.037601, 1.742469)
Slope	3.221	0.356	(2.522384, 3.919848)

Theoretical Spontaneous Response Rate = 0.000

Estimated EC Values and Confidence Limits

A. Point		Lower Conc.	Upper 95% Confidence Limits
EC 1.00	**3.6763**	**2.3057**	**5.0567**
EC 5.00	**5.9835**	**4.2105**	**7.6967**
EC10.00	7.7579	5.7677	9.6905
EC15.00	9.2441	7.1030	11.3677
EC50.00	**19.3918**	**16.0216**	**23.8609**
EC85.00	40.6789	32.0109	66.5425
EC90.00	48.4722	37.3312	70.0429
EC95.00	62.8463	46.7199	96.5266
EC99.00	102.2872	70.6694	177.37

Experiment 2 GKE

		Observed	Adjusted	Predicted
	Conc.	Number Exposed	Number Resp.	Proportion Responding	Proportion Responding
Control	45	1	0.022	0.000	0.028
10.0000	46	7	0.152	0.128	0.079
15.0000	45	7	0.156	0.131	0.187
20.0000	44	15	0.341	0.322	0.301
25.0000	39	17	0.436	0.420	0.407
30.0000	43	18	0.419	0.402	0.499
35.0000	38	22	0.579	0.567	0.578
40.0000	42	30	0.714	0.706	0.643
50.0000	43	33	0.767	0.761	0.743

Chi - Square Heterogeneity = 4.305

Mu = 1.478
Sigma = 0.339

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	0.635	0.625	(-0.591, 1.861)
Slope	2.954	0.431	(2.110, 3.798)
Spontaneous Response Rate	0.028	0.024	(-0.019, 0.075)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	**4.90**	**2.34**	**7.44**
EC 5.00	**8.33**	**4.89**	**11.32**
EC10.00	11.06	7.23	14.20
EC15.00	13.39	9.39	16.58
EC50.00	**30.04**	**26.17**	**34.53**
EC85.00	67.38	54.24	96.81
EC90.00	81.57	63.31	125.75
EC95.00	108.28	79.39	185.82
EC99.00	184.16	120.78	388.36

Experiment 3 GKE

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	34	4	0.118	0.000	0.119
1.0000	32	2	0.062	-.062	0.000
3.0000	33	6	0.182	0.073	0.000
10.0000	38	7	0.184	0.075	0.000
30.0000	30	30	1.000	1.000	1.000
40.0000	34	34	1.000	1.000	1.000
50.0000	38	38	1.000	1.000	1.000
75.0000	41	41	1.000	1.000	1.000
100.0000	35	35	1.000	1.000	1.000

Chi - Square Heterogeneity = 42.380

Mu = 1.446
Sigma = 0.000

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	%-6940809.000	%1550442.000	(%-10734740.000, %-3146877.500)
Slope	%4799654.500	%651974.250	(%3204273.500, %6395035.500)
Spontaneous Response Rate	0.118	1.084	(-2.534, 2.769)

Estimated EC Values and Confidence Limits

Point	Conc.	95% Lower Confidence Limits	95% Upper Confidence Limits
EC 1.00	**27.932**	**5.7504**	**147.9063**
EC 5.00	**27.932**	**5.7504**	**147.9065**
EC10.00	27.9323	5.7504	147.9065
EC15.00	27.9323	5.7504	147.9065
EC50.00	**27.9323**	**5.7504**	**147.9066**
EC85.00	27.9323	5.7504	147.9066
EC90.00	27.9323	5.7504	147.9066
EC95.00	27.9323	5.7504	147.9066
EC99.00	27.9324	5.7504	147.9068

Experiment 4 GKE

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	36	5	0.139	0.000	0.133
1.0000	33	3	0.091	-048	0.000
3.0000	37	7	0.189	0.065	0.020
10.0000	40	19	0.475	0.395	0.410
30.0000	38	36	0.947	0.939	0.925
40.0000	40	38	0.950	0.942	0.970
50.0000	40	40	1.000	1.000	0.987
75.0000	40	40	1.000	1.000	0.998
100.0000	38	38	1.000	1.000	1.000

Chi - Square Heterogeneity = 2.522

Mu = 1.065
Sigma = 0.286

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	1.279	0.721	(-0.134, 2.693)
Slope	3.494	0.547	(2.422, 4.566)
Spontaneous Response Rate	0.133	0.036	(0.063, 0.202)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	**2.5064**	**0.9571**	**4.2489**
EC 5.00	**3.9272**	**1.8125**	**6.0472**
EC10.00	4.9895	2.5422	7.3148
EC15.00	5.8646	3.1901	8.3280
EC50.00	**116110**	**8.1290**	**14.7647**
EC85.00	22.9879	18.4204	29.4357
EC90.00	27.0195	21.6756	35.7395
EC95.00	34.3288	27.0955	48.5059
EC99.00	53.7878	39.7967	89.0081

Experiment 5 GKE

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	87	4	0.046	0.000	0.036
3.0000	41	1	0.024	-.012	0.000
10.0000	39	1	0.026	-.011	0.000
30.0000	36	12	0.333	0.308	0.283
50.0000	41	30	0.732	0.722	0.756
100.0000	44	44	1.000	1.000	0.992

Chi - Square Heterogeneity = 0.980

Mu = 1.578
Sigma = 0.175

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-3.999	1.753	(-7.436, -0.564)
Slope	5.704	1.077	(3.594, 7.815)
Spontaneous Response Rate	0.036	0.014	(0.008, 0.064)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.0	**14.79**	**7.86**	**19.98**
EC 5.00	**19.47**	**12.08**	**24.59**
EC10.00	22.54	15.16	27.52
EC15.00	24.89	17.65	29.73
EC50.00	**37.81**	**32.31**	**42.82**
EC85.00	57.46	49.96	73.01
EC90.00	63.43	54.26	84.55
EC95.00	73.45	60.99	105.64
EC99.00	96.71	75.35	161.76

Experiment 6 GKE

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	101	1	0.010	0.000	0.033
1.0000 39	1	0.026	-.007	0.000	
3.0000 39	4	0.103	0.072	0.000	
10.0000	35	1	0.029	-.004	0.005
30.0000	37	8	0.216	0.110	0.149
50.0000	34	11	0.323	0.301	0.369
100.0000	42	32	0.762	0.754	0.734

Chi - Square Heterogeneity = 7.293

Mu = 1.804
Sigma = 0.314

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-0.737	1.145	(-2.981, 1.508)
Slope	3.180	0.643	(1.919203, 4.442)
Spontaneous Response Rate	0.033	0.012	(0.008166, 0.057)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	**11.813**	**3.967**	**19.247**
EC 5.00	**19.346**	**8.877**	**27.735**
EC10.00	25.167	13.571	33.866
EC15.00	30.055	18.002	38.908
EC50.00	**63.648**	**51.948**	**80.016**
EC85.00	134.787	101.077	244.010
EC90.00	160.969	115.800	324.574
EC95.00	209.397	141.068	497.376
EC99.00	342.938	202.8747	1115.167

Experiment 7 IKE

Conc.	Number Exposed	Number Resp.		Observed Proportion Responding		Adjusted Proportion Responding		Predicted Proportion Responding
Control	87	4		0.046		0.000		0.050
1.0000	42	2		0.048		-.003		0.000
3.0000	46	1		0.022		-.030		0.000
10.0000	40	4		0.100		0.052		0.002
30.0000	41	20	0.488		0.461		0.441	
50.0000	31	25	0.807		0.796		0.864	
75.0000	44	44	1.000		1.000		0.981	
100.0000	42	42	1.000		1.000		0.997	

Chi - Square Heterogeneity = 4.676

Mu = 1.503
Sigma = 0.178

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-3.439	1.616	(-6.606, -0.272)
Slope	5.613	1.005	(3.643, 7.583)
Spontaneous Response Rate	0.050	0.015	(0.021, 0.080)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	**12.28**	**6.36**	**17.0058**
EC 5.00	**16.23**	**9.73**	**21.030**
EC10.00	18.84	12.19	23.589
EC15.00	20.84	14.17	25.514
EC50.00	**31.88**	**26.23**	**36.349**
EC85.00	48.77	42.86	58.671
EC90.00	53.93	46.94	67.381
EC95.00	62.60	53.25	83.4556
EC99.00	82.79	66.52	126.406

Experiment 10 IKE

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	100	4	0.040	0.000	0.038
1.0000	41	1	0.024	-.015	0.000
3.0000	40	1	0.025	-.014	0.000
10.0000	42	2	0.048	0.010	0.000
30.0000	38	18	0.474	0.453	0.443
50.0000	40	36	0.900	0.896	0.912
75.0000	40	40	1.000	1.000	0.994
100.0000	39	39	1.000	1.000	1.000

Chi - Square Heterogeneity = 0.641

Mu = 1.498
Sigma = 0.149

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-5.087	2.057	(-9.119217, -1.054522)
Slope	6.732 1	0.303	(4.178942, 9.285885)
Spontaneous Response Rate	0.038	0.0143	(0.010429, 0.066402)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	**14.21**	**7.72**	**18.88**
EC 5.00	**17.94**	**11.18**	**22.47**
EC10.00	20.32	13.61	24.68
EC15.00	22.10	15.52	26.33
EC50.00	**31.50**	**26.48**	**35.31**
EC85.00	44.90	40.03	53.46
EC90.00	48.82	43.14	60.35
EC95.00	55.28	47.83	72.76
EC99.00	69.79	57.37	104.55

Textile effluent

Experiment 1 GTE

Conc.	Number Exposed	Observed Number Resp.	Adjusted Proportion Responding	Predicted Proportion Responding	Proportion Responding
Control	32	2	0.062	0.000	0.070
1.0000	32	2	0.062	-.009	0.000
3.0000	36	3	0.083	0.014	0.000
5.0000	35	2	0.057	-.014	0.000
10.0000	35	3	0.086	0.017	0.009
20.0000	35	15	0.429	0.385	0.283
30.0000	28	15	0.536	0.500	0.680
40.0000	31	27	0.871	0.861	0.886
50.0000	32	32	1.000	1.000	0.962
60.0000	40	40	1.000	1.000	0.988
75.0000	37	37	1.000	1.000	0.998
100.0000	35	35	1.000	1.000	1.000

Chi - Square Heterogeneity = 7.273

Mu = 1.398
Sigma = 0.169

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-3.270	1.299	(-5.816, -0.724)
Slope	5.915	0.863	(4.224, 7.606)
Spontaneous Response Rate	0.070	0.020	(0.031, 0.110)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	**10.11**	**6.28**	**13.28**
EC 5.00	**13.18**	**9.06**	**16.40**
EC10.00	15.19	11.00	18.38
EC15.00	16.71	12.53	19.87
EC50.00	**25.01**	**21.36**	**28.06**
EC85.00	37.44	33.52	43.04
EC90.00	41.19	36.65	48.47
EC95.00	47.44	41.52	58.21
EC99.00	61.86	51.81	83.14

Experiment 4 GTE

Conc.	Number Exposed	Number Resp.	Observed Proportion Responding	Adjusted Proportion Responding	Predicted Proportion Responding
Control	26	3	0.115	0.000	0.177
1.0000	27	5	0.185	0.010	0.005
3.0000	30	14	0.467	0.352	0.159
5.0000	29	12	0.414	0.288	0.394
10.0000	28	20	0.714	0.653	0.764
15.0000	26	23	0.885	0.866	0.903
20.0000	29	29	1.000	1.000	0.956
25.0000	33	33	1.000	1.000	0.979
30.0000	31	31	1.000	1.000	0.989
50.0000	33	33	1.000	1.000	0.999

Chi - Square Heterogeneity = 8.373

Mu = 0.781
Sigma = 0.304

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	2.435	0.529	(1.397, 3.473)
Slope	3.283	0.516	(2.273, 4.294)
Spontaneous Response Rate	0.177	0.053	(0.072, 0.281)

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	**1.18**	**0.42**	**2.06**
EC 5.00	**1.91**	**0.84**	**3.00**
EC10.00	2.46	1.21	3.68
EC15.00	2.92	1.54	4.22
EC50.00	**6.04**	**4.18**	**7.74**
EC85.00	12.50	9.96	16.16
EC90.00	14.84	11.83	19.90
EC95.00	19.15	14.97	27.61
EC99.00	30.88	22.46	52.88

Experiment 8 GTE

Conc.	Number Exposed	Observed Number Resp.		Adjusted Proportion Responding		Predicted Proportion Responding		Proportion Responding
1.0000	35	2		0.057		0.025		0.000
3.0000	34	2		0.059		0.026		0.000
5.0000	35	3		0.086		0.054		0.000
10.0000	33	5		0.151		0.122		0.052
15.0000	29	9		0.310		0.287		0.337
20.0000	35	21	0.600		0.586		0.667	
30.0000	32	32	1.000		1.000		0.949	
50.0000	40	40	1.000		1.000		0.999	
100.0000	37	37		1.000		1.000		1.000

Chi - Square Heterogeneity = 9.201

Mu = 1.238
Sigma = 0.147

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-3.441	1.367	(-6.120, -0.762)
Slope	6.819	1.079	(4.705, 8.934)

Theoretical Spontaneous Response Rate = 0.033

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	**7.88**	**5.32**	**9.77**
EC 5.00	**9.92**	**7.38**	**11.72**
EC10.00	11.22	8.77	12.94
EC15.00	12.18	9.84	13.85
EC50.00	**17.29**	**15.52**	**19.05**
EC85.00	24.53	21.93	29.25
EC90.00	26.65	23.52	32.75
EC95.00	30.13	26.02	38.85
EC99.00	37.92	31.25	53.80

Trimmed Spearman-Karber analyses

DATE: 20-08-98 **TEST NUMBER: EXP 6**
CHEMICAL: KRAFT EFFLUENT **SPECIES: TRICORYTHUS**

RAW DATA:

CONCENTRATION(%)	1.00	3.00	10.00	30.00	50.00	100.00
NUMBER EXPOSED:		39	39	35	37	34	42

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
96	67.81	52.73	87.22	24.82

DATE: 17-08-98 **TEST NUMBER: EXP 7**
CHEMICAL: KRAFT EFFLUENT **SPECIES: TRICORYTHUS**

RAW DATA:

CONCENTRATION(%)	1.00	3.00	10.00	30.00	50.00	75.00	100.00
NUMBER EXPOSED:		42	46	40	41	31	44	42

DURATION (hours)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
96	27.16	22.28	33.11	.00

DATE: 17-08-98 **TEST NUMBER: EXP 8**
CHEMICAL: KRAFT EFFLUENT **SPECIES: TRICORYTHUS**

RAW DATA:

CONCENTRATION(%)	1.00	3.00	10.00	30.00	50.00	75.00	100.00
NUMBER EXPOSED:		39	38	38	39	42	44	41

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
96	28.71	24.63	33.46	.00

DATE: 20-08-98 **TEST NUMBER: EXP 9**
CHEMICAL: KRAFT EFFLUENT **SPECIES: TRICORYTHUS**

RAW DATA:

CONCENTRATION(%)	1.00	3.00	30.00	50.00	75.00	100.00
NUMBER EXPOSED:		39	40	40	43	40	40

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
96	19.32	13.57	27.51	11.66

DATE: 20-08-98 **TEST NUMBER: EXP 10**
CHEMICAL: KRAFT EFFLUENT **SPECIES: TRICORYTHUS**

RAW DATA:

CONCENTRATION(%)	1.00	3.00	10.00	30.00	50.00	75.00	100.00
NUMBER EXPOSED:		41	40	42	38	40	40	39

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
96	27.66	23.26	32.90	.00

DATE: 24-11-1997 **TEST NUMBER: EXP1**
CHEMICAL: TEXTILE EFFLUENT **SPECIES: BAETIDS**

RAW DATA:

CONCENTRATION(%)	1.00	3.00	5.00	10.00	20.00	30.00	40.00	50.00	75.00	100.00
NUMBER EXPOSED:	32	36	35	35	35	28	31	32	37	35

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
24	25.02	22.12	28.31	.00
48	23.08	19.64	27.11	.00
96	23.61	19.00	29.34	.00

DATE: 1 -5-1998 **TEST NUMBER: EXP 2**
CHEMICAL: TEXTILE EFFLUENT **SPECIES: BAETIDS**

RAW DATA:

CONCENTRATION (%)	1.00	3.00	10.00	30.00	40.00	50.00	100.00
NUMBER EXPOSED:	21	21	23	21	29	28	29

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
96	6.40	2.80	14.63	26.67
48	9.19	5.72	14.77	18.81
24	11.20	7.83	16.04	10.20

DATE: 7 -5-1998 **TEST NUMBER: EXP3**
CHEMICAL: EFFLUENT **SPECIES: BAETIDS**

RAW DATA:

CONCENTRATION (%)	1.00	3.00	10.00	15.00	20.00	25.00	30.00	50.00
NUMBER EXPOSED:	24	25	23	26	29	28	27	29

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
24	10.60	8.86	12.68	.00
48	9.92	8.10	12.16	4.08
96	9.43	7.49	11.86	8.16

DATE: 11-5-199 **TEST NUMBER: EXP4**
CHEMICAL: TEXTILE EFFLUENT **SPECIES: BAETIDS**

RAW DATA:

CONCENTRATION (%)	1.00	3.00	5.00	10.00	15.00	20.00	25.00	30.00	50.00
NUMBER EXPOSED:	27	31	25	25	26	32	34	32	35

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
24	10.68	8.81	12.96	.00
48	7.19	5.46	9.45	3.70
96	5.58	4.08	7.64	7.72

DATE: 24-11-1998 **TEST NUMBER: EXP 8**
CHEMICAL: TEXTILE EFFLUENT **SPECIES: BAETIDS**

RAW DATA:

CONCENTRATION(%)	1.00	3.00	10.00	20.00	30.00	40.00	50.00	60.00	75.00	100.00
NUMBER EXPOSED:		32	35	35	36	36	33	32	40	37	35

DURATION (HOURS)	LC50	LOWER 95% LIMIT	UPPER 95% LIMIT	PERCENT TRIM
24	26.66	23.93	29.71	.00
48	29.65	25.50	34.48	.00
96	30.74	26.41	35.79	.00

APPENDIX C

Hazard-based guideline ranges for kraft effluent

TABLE C 1

EXPERIMENT 2, GKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2) AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1)AND HAZARD-BASED DESCRIPTION (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN, IN PRESS.

Tolerance test end-points	% effluent concentration	Summarised risk description	River health class	Suggested effluent concentration
Chronic test results not available	Unknown	minimal hazard to intolerant biota - no acute response	A	0 – 2
AEV LC1 lower 95% CL LC1	2.4 2.3 4.9	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	2 – 5
LC5 lower 95% CL LC1 upper 95% CL	4.8 7.4	moderate hazard to intolerant biota, 95% probability of mortality between 1-5 % after acute exposure.	C	5 – 7
LC5	8.3	High hazard - best estimate of 5% mortality after acute exposure.	D	7 – 11
LC5 upper 95% CL	11.3	Unacceptable hazard – 95% probability of at least 5% mortality after acute exposure.	E/F	>11

TABLE C 2

EXPERIMENT 3, GKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2) AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1)AND HAZARD-BASED DESCRIPTION (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN, IN PRESS.

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 response	A	0 - 0.5
AEV LC1 lower 95% CL LC1	0.5 0.01 1.1	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	0.5 – 1
LC5 lower 95% CL LC1 upper 95% CL	0.04 3.6	moderate hazard to intolerant biota, 95% probability of mortality between 1-5 % after acute exposure.	C	1 – 4
LC5	2.1	High hazard - best estimate of 5% mortality after acute exposure.	D	4 – 6
LC5 upper 95% CL	5.4	Unacceptable hazard – 95% probability of at least 5% mortality after acute exposure.	E/F	>6

TABLE C 3

EXPERIMENT 4, GKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2) AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1)AND HAZARD-BASED DESCRIPTION (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN, IN PRESS.

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 response	A	0-1
AEV LC1 lower 95% CL LC1	1.2 0.9 2.5	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	1 - 3
LC5 lower 95% CL LC1 upper 95% LC	1.8 4.2	moderate hazard to intolerant biota, 95% probability of mortality between 1-5 % after acute exposure.	C	3 - 4
LC5	3.9	High hazard - best estimate of 5% mortality after acute exposure.	D	4 - 6
LC5 upper 95% CL	6	Unacceptable hazard – 95% probability of at least 5% mortality after acute exposure.	E/F	>6

TABLE C 4

EXPERIMENT 5, GKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2) AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1)AND HAZARD-BASED DESCRIPTION (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN, IN PRESS.

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 response	A	0 – 7
AEV LC1 lower 95% CL LC1	7.3 7.8 14.7	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	7 – 15
LC5 lower 95% CL LC1 upper 95% CL	12 19.9	moderate hazard to intolerant biota, 95% probability of mortality between 1-5 % after acute exposure.	C	15 – 20
LC5	19.4	High hazard - best estimate of 5% mortality after acute exposure.	D	20 – 25
LC5 upper 95% CL	24.5	Unacceptable hazard - 95% probability of at least 5% mortality after acute exposure.	E/F	>25

TABLE C 5

EXPERIMENT 6, GKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2) AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1)AND HAZARD-BASED DESCRIPTION (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN, IN PRESS.

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 response	A	0 – 6
AEV LC1 lower 95% CL LC1	5.9 3.9 11.8	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	6 – 12
LC5 lower 95% CL LC1 upper 95%CL	8.8 19.2	moderate hazard to intolerant biota, 95% probability of mortality between 1-5 % after acute exposure.	C	12 – 20
LC5	19.3	High risk - best estimate of 5% mortality after acute exposure.	D	20 – 30
LC5 upper 95% CL	27.7	Unacceptable risk - 95% probability of at least 5% mortality after acute exposure.	E/F	>30

TABLE C 6

EXPERIMENT 7, IKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2) AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1)AND HAZARD-BASED DESCRIPTION (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN, IN PRESS.

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 response	A	0 - 6
AEV LC1 lower 95% CL LC1	6.1 6.3 12.2	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	6 - 12
LC5 lower 95% CL LC1 upper 95% CL	9.7 17	Moderate hazard to intolerant biota, 95% probability of mortality between 1-5 % after acute exposure.	C	12 - 17
LC5	16.2	High hazard - best estimate of 5% mortality after acute exposure.	D	17 -26
LC5 upper 95% CL	26.2	Unacceptable hazard – 95% probability of at least 5% mortality after acute exposure.	E/F	>26

TABLE C 7

EXPERIMENT 10, GKE: A RANKED LIST OF TOXICITY TEST END-POINTS, EACH WITH A SPECIFIC HAZARD DESCRIPTION (TABLE 2.2) AND ASSOCIATED RIVER HEALTH CLASS. RESULTANT GUIDELINE RANGES FOR KRAFT EFFLUENT ARE GIVEN. CLASS DEFINITIONS (TABLE 2.1)AND HAZARD-BASED DESCRIPTION (TABLE 2.2) ARE BASED ON PALMER AND SCHERMAN, IN PRESS.

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 response	A	0 - 7
AEV LC1 lower 95% CL LC1	7.1 7.7 14.2	Low hazard to moderate biota: evidence of an acute response, but 95% probability of less than 1% mortality after acute exposure	B	7 - 14
LC5 lower 95% CL LC1 upper 95% CL	11.2 18.9	moderate hazard to intolerant biota, 95% probability of mortality between 1-5 % after acute exposure.	C	14 - 18
LC5	17.9	High hazard – best estimate of 5% mortality after acute exposure.	D	18 - 23
LC5 upper 95% CL	22.5	Unacceptable hazard – 95% probability of at least 5% mortality after acute exposure.	E/F	>23

APPENDIX D

Physico-chemical constituents of groundwater

TABLE D 1

RANGES OF PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENT CONCENRATIONS (EXPRESSED IN MG /L) MONITORED DURING GRO UNDWATER TOXICITY TE STING EXPERIMENT 11. (SAMPLES WERE ANALYZED BY IWQS).

Parameter (mg/l)	Groundwater mixtures (%)				Control
	10	25	50	100	
EC (mS/m)	27.9-31.3	43.3-51.3	74.3-77.4	107.4-143.0	11.7-15.4
TDS	173.0-215.0	291.0-331.0	500.0-569.0	814.0-871.0	96.0-108.0
pH	7.9-8.1	8.1-8.2	8.3-8.4	8.3-8.4	7.9-8.0
SO_4^{2-}	20.0-49.0	43.0-56.0	78.0-94.0	144.0-160.0	9.0-12.0
Cl^-	<25.0-32.0	47.0-69.0	122.0-142.0	227.0-259.0	<25.0
Ca^{2+}	18.0-21.0	32.0-35.0	56.0-61.0	71.0-76.0	9.0-10.0
Mg^{2+}	11.0-13.0	19.0-21.0	32.0-37.0	62.0-69.0	6.0-7.0
Na^+	14.0-16.0	27.0-29.0	50.0-57.0	95.0-103.0	7.0-8.0
NH_4^+ - N	<0.04	<0.04	<0.04	<0.04	<0.04
$NO_3^- + NO_2^-$ - N	<0.04	<0.04	0.0-0.10	<0.04	<0.04
PO_4^{3-} -P	0.1-0.3	0.2-0.4	0.4-0.6	0.6-1.0	0.0-0.2

TABLE D 2
RANGES OF PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENT CONCENRATIONS (EXPRESSED IN MG /L) MONITORED DURING GROUNDWATER TOXICITY TESTING EXPERIMENT 12.
(SAMPLES WERE ANALYZED BY IWQS).

Parameter (mg/l)	Groundwater mixtures (%)				Control
	10	25	50	100	
EC (mS/m)	25.1-30.6	47.6-53.7	71.7-84.2	109.8-144.0	11.7-15.4
TDS	177.0-204.0	288.0-333.0	499.0-556.0	820.0-881.0	96.0-108.0
pH	7.9-8.1	7.9-8.2	8.2.0-8.3.0	8.2-8.3	7.9-8.0
SO_4^{2-}	20.0-25.0	39.0-46.0	81.0-85.0	150.0-166.0	9.0-12.0
Cl^-	< 25.0-33.0	61.0-73.0	120.0-139.0	233.0-264.0	<25.0
Ca^{2+}	18.0-21.0	32.0-37.0	55.0-60.0	70.0-98.0	9.0-10.0
Mg^{2+}	11.0-13.0	19.0-22.0	32.0-37.0	61.0-68.0	6.0-7.0
Na^+	15.0-16.0	26.0-31.0	50.0-54.0	95.0-104.0	7.0-8.0
NH_4^+ - N	<0.04	<0.04	<0.04	<0.04	<0.04
NO_3^- + NO_2^- - N	<0.04	<0.04	<0.04	<0.04	<0.04
PO_4^{3-} -P	0.1-0.3	0.3-0.4	0.4-0.6	0.5-1.1	0.0-0.2

TABLE D 3

PHYSICO-CHEMICAL CONSTITUENTS AND NUTRIENT CONCENTRATIONS (EXPRESSED IN MG/L) MONITORED DURING GROUNDWATER TOXICITY TESTING SHOWING CHANGES DURING TOXICITY TESTING OVER 12 DAYS.

Parameter (mg/l)	Day 0		Day 4		Day 8		Day 12	
	Experiment 11	Experiment 12	Experiment 11	Experiment 12	Experiment 11	Experiment 12	Experiment 11	Experiment 12
EC (mS/m)	139.0	142.0	107.0	109.0	143.0	144.0	115.0	139.0
TDS	871.0	881.0	814.0	828.0	837.0	847.0	818.0	820.0
pH	8.4	8.3	8.3	8.3	8.3	8.2	8.3	8.3
SO_4^{2-}	144.0	150.0	160.0	166.0	160.0	161.0	157.0	158.0
Cl^-	227.0	233.0	252.0	262.0	259.0	264.0	253.0	256.0
Ca^{2+}	94.0	98.0	73.0	72.0	76.0	75.0	71.0	70.0
Mg^{2+}	62.0	61.0	63.0	65.0	65.0	66.0	69.0	68.0
Na^+	95.0	95.0	102.0	104.0	103.0	105.0	100.0	100.0
NH_4^+ - N	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
$NO_3^- + NO_2^-$ - N	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
PO_4^{3-} - P	1.0	1.0	0.9	1.1	0.6	0.5	0.9	0.8