THE ROLE OF ACUTE TOXICITY DATA FOR SOUTH AFRICAN FRESHWATER MACROINVERTEBRATES IN THE DERIVATION OF WATER QUALITY GUIDELINES FOR SALINITY

A thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

of

RHODES UNIVERSITY

By

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January 2005

Abstract

Water resources are under ever-increasing pressure to meet the demands of various water users both nationally and internationally. The process of anthropogenically-induced salinisation serves to exacerbate this pressure by limiting the quantity and quality of water available for future use. Water quality guidelines provide the numerical goals which water resource managers can use to adequately manage and protect aquatic ecosystems. Various methods which have been developed and used internationally to derive such guidelines are discussed.

Acute toxicity tests were conducted using two inorganic salts, NaCl and Na_2SO_4. Field collected, indigenous, freshwater macroinvertebrates were used as tests organisms. Data generated from these tests contributed to the expansion of the currently limited toxicological database of response data for indigenous organisms and the suitability of using such organisms for future testing was discussed. Salt sensitivities of indigenous freshwater invertebrates were compared those of species sourced from an international toxicological database and were found to have similar ranges of tolerances to NaCl and Na_2SO_4.

Species sensitivity distributions (SSDs), a method of data extrapolation, were derived using different types of toxicological data, and hence different guideline values or protective concentrations were derived. These concentrations were equated to boundary values for South Africa's ecological Reserve categories, which are used to describe degrees of health for aquatic ecosystems. Provisional results suggest that using only acute toxicity data in guideline derivation provides ecosystem protection that is under-protective. Chronic toxicity data, which include endpoints other than mortality, provide the most realistic environmental protection but lack data confidence due to small sample sizes (acute tests are more readily conducted than chronic tests). The potential contribution of sub-chronic data to guideline derivation is highlighted as these data are more readily extrapolated to chronic endpoints than acute data and sub-chronic tests are not as complex and demanding to conduct as chronic tests.

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Acknowledgements

With sincere thanks to the Water Research Commission for generous funding and the Unilever Centre for Environmental Water Quality (UCEWQ), Grahamstown, for additional funding. Thank you to Professor Tally Palmer for your immense patience, ongoing assistance and support. You have been a role model to me through your courage, determination and strength of character. Dr Nikite Muller and Dr Heather Davies-Coleman for further assistance and academic support. The staff of the UCEWQ: Lora Pakhomova, Tarqyn Human, Makatleho ('Valisa') and Andrew Gordon for many hours of laboratory and technical assistance as well as the students of UCEWQ, Nosiphiwo Ketse, Sekhonyana Leratoli, Pumza Maseti and especially Andrew Slaughter for both laboratory and field assistance. Gordon O'Brien for academic advice. My deepest gratitude to Leanne Du Preez and Sheril Daniel for your loyal friendship, ongoing patience with me and emotional support. To my parents for believing in me and standing by me. To Jesus for providing me the opportunity and making it possible the whole way through.

"*My grace is sufficient for you, for my power is made perfect in your weakness"*, Romans 12 v 9

List of commonly used abbreviations

A&NZ	Australia and New Zealand
ACR	Acute to Chronic Ratio
AF	Assessment Factor
ANZECC	Australian and New Zealand Environment and Conservation Council
ANZG	Australia and New Zealand Guidelines
AQUIRE	Aquatic toxicity information retrieval
BBM	Building Block Methodology
BT	Burr Type
BV	Boundary Value
CCME	Canadian Council of Ministers of the Environment
CCREM	Canadian Council of Resource and Environmental Ministers
CSIR	Council for Scientific and Industrial Research
CWQG	Canadian Water Quality Guidelines
DO	Dissolved Oxygen
DRIFT	Downstream Response to Instream Flow Transformations
DWAF	Department of Water Affairs and Forestry
EC	Effect Concentration
EC	Electrical Conductivity
ESD	Ecological Sustainable Development
EWQ	Environmental Water Quality
IFR	Instream Flow Requirement
IWR	Institute for Water Research
LC	Lethal Concentration
LC_{50}	Lethal Concentration that kills 50% of the population observed
LCL	Lower Confidence Limit
LOEC	Lowest Observed Effect Concentration
LRA	Linear Regression Analysis
MATC	Maximum Acceptable Toxicant Concentration
NOEC	No Observed Effect Concentration
NR-LETH	Lethal: 100% mortality or 0% survival.
NR-ZERO	Zero mortality: 0% mortality or 100% survival of organisms.
NWA	National Water Act
NWRS	National Water Resource Strategy
OECD	Organisation for Economic and Co-operation and Development
PC	Protective Concentration
PES	Present Ecological State
ppm	Parts per million
RAU	Rand Afrikaans University
RDM	Resource Directed Measures
RF	Range Finding
RQO	Resource Quality Objective
RQS	Resource Quality Services
SAWQGs	South African Water Quality Guidelines
SDC	Source Directed Control
SSD	Species Sensitivity Distribution
TDS	Total Dissolved Salts/solids
TIMS	Toxicologically Important Major Salt
TSK	Trimmed-Spearman Kärber
TV	Trigger Value
TWQR	Target Water Quality Range
UCEWQ	Unilever Centre for Environmental Water Quality
UCL	Upper Confidence Limit
USEPA	United States Environmental Protection Agency
WQG	Water Quality Guideline

CHAPTER 1: Introduction

Demands on aquatic ecosystems are numerous and include those made by industry, agriculture, and domestic users for basic human needs such as drinking water and sanitation. Aquatic ecosystems also provide important services such as the retention, supply and transport of water by means of rivers, estuaries, lakes, dams, wetlands and aquifers, all of which are part of the hydrological cycle. Other services include the dilution, removal and purification of wastes; commercial and subsistence supply products such as fish and plants; and sports and recreation facilities (Palmer, 1999). Supplies of freshwater are limited worldwide and demands on freshwater resources are increasing (Davies and Day, 1998a) due to rising populations and growing industrial and agricultural activity. Of the 72 per cent of the earth's surface that is constituted by water (Barnes, 1980), only 2.53 per cent is freshwater (Newson, 1994). Problems with supply are often compounded by the naturally inequitable geographic distribution of water both spatially and temporally (Davies and Day, 1998a; Gaylard *et al*., 2003).

Added to the challenges of water supply are those associated with water quality, which is also frequently impacted. The deterioration of both water quality and quantity may be exacerbated by natural variability and changing climatic conditions such as drought. This variability has resulted in a paradigm of managing natural resources so as to provide a reliable supply of good quality water, which in turn has driven development and a focus on engineering solutions. The main approach to water resource management has been to build dams so as to regulate supply. Dams have negative ecological consequences, disrupting aquatic ecosystems by severely modifying flow regimes of rivers which in turn modify natural structures of aquatic fauna and flora. However, the emerging field of holistic methodologies such as environmental flow assessment (Tharme, 2002), has led to a more comprehensive understanding of the negative impacts that impoundments have on aquatic ecosystems (O'Keeffe *et al*., 1992). These efforts have focused on better management of dam releases in an attempt to maximise aquatic ecosystem health. In the process of developing methods for managing aquatic ecosystems in South Africa, most of the method development has focused primarily on rivers, and since rivers are such important freshwater systems (Rabie and Day, 1992), they form the focus for this study.

Often the urgent need for rivers to provide more water-related services conflicts with the need to improve or maintain the ecology of the country's rivers (King and Louw,

1998). Water resource managers are under pressure to find equilibrium in managing and meeting the demands of the country's water while at the same time, adequately protecting the resource. This has prompted a change in paradigm to one that still appreciates growing demands of water supply, but also acknowledges that aquatic resources are limited and that ecosystem health is deteriorating. Aquatic ecosystems have been recognised, not as users of water in competition with other users, but rather as the base of the resource itself that needs to be actively cared for if development is to be sustainable (DWAF, 1997, King and Louw, 1998). Allanson *et al*. (1990) support this paradigm shift: "the general conservation of rivers is complicated by their longitudinal nature, by their vulnerability to catchment changes, and by their importance as scarce water resources. Hence, the aim of river conservation cannot therefore be to preserve them in pristine condition, but rather to maintain them as renewable natural resources, to be exploited within limits for sustainable yields, and for multiple purposes". One of the greatest challenges riverine ecologists face today is adequately communicating their knowledge of spatial and temporal variability of rivers to managers in ways that enable managers to develop appropriate approaches to management (Rogers and O'Keeffe, 2003). An interdisciplinary understanding of river ecology is essential if this communication between scientists and managers is to be successfully achieved (Rogers and O'Keeffe, 2003).

As paradigms have changed, the tools used in water resource management have evolved. In apartheid South Africa, the Water Act (No. 54 of 1956) was based on the principle of 'riparianity', where the right to use water was based on land ownership (Palmer, 1999) and it focused primarily on inland running water (O'Keeffe, *et al*., 1992). Because land ownership was discriminatory, a privileged few had majority access to water. In 1994, with the emergence of democracy in the country, the principle of ripariarinty was challenged and a new water law was envisaged. To guide the process of developing new legislation, widespread public participation and consultation culminated in an outline of key principles, on which the new water law would be based. These principles provided the foundation for the 'White Paper on a National Water Policy for South Africa' (DWAF, 1997), which was based on two central principles: equity and sustainability (DWAF, 1996b; Palmer *et al*., 2002). The White Paper formed the policy basis for the new legal framework for water resource management, the National Water Act, No. 36 of 1998 (Palmer *et al*., 2002). Sustainability and equity remain as central guiding principles "in the protection, use, development, conservation, management and control of water resources" for the country (Chapter 1, National Water Act, No. 36 of 1998).

The new Act makes provision for both a “basic human needs Reserve” (that quantity and quality of water which provides for essential needs of individuals) and an “ecological Reserve” (that quantity and quality of water required to protect the ecosystems that comprise the water resource). The Reserve is viewed as one of the main policy and legal tools to ensure equity and sustainability. The National Water Act states that “the Minister, the Director-General, an organ of state and a water management institution, must give effect to the Reserve as determined in terms of this Part [the Reserve] when exercising any power or performing any duty in terms of the Act” (NWA, 1998).

With a conceptual basis for resource management in place (the White paper) and the establishment of a legal framework (the National Water Act), the need arose for an implementation strategy that would ensure action towards resource management. In the Integrated Manual for Resource Directed Measures for Protection of Water Resources (DWAF, 1999, DWAF, 2003), the Department of Water Affairs and Forestry (DWAF) identified three main components of policy implementation:

1. Protection to ensure sufficient water quantity and water quality (especially in relation to human health), to meet basic human needs.
2. Protection of ecosystem structure and function, in order to ensure that utilisation of water resources can be sustained on a long term basis.
3. Meeting of water quality requirements for other water users (agriculture, industry, recreation) as far as possible, within the constraints of requirements for protection of basic human needs and the protection of water resources.

These components further highlight the need for water resource managers to adopt an integrated and adaptive approach to management. The aim of this approach is to ensure sustainable, equitable and efficient use of the country’s aquatic resources. This is set out in the draft National Water Resource Strategy (NWRS) (DWAF, 2002). Essentially, the strategy acts as the implementation framework for the National Water Act (No. 36 of 1998) and outlines the goals and objectives of water resource management for the country. It also provides plans, guidelines and strategies on how to achieve these goals.
In order for these new holistic ideas to be practically implementable, the Department of Water Affairs has identified four regulatory activities (DWAF, 1999):

Firstly, **Resouce-directed measures (RDM)**, involve co-operatively defining the appropriate level of protection for a water resource, and on that basis, setting clear numerical or descriptive goals for the resource quality of the resource i.e. Resource

Quality Objectives (RQOs). Secondly, **Source-directed controls (SDC)**, require controlling impacts on the water resource through the use of regulatory measures such as registration, permits, directives and prosecution, and economic incentives such as levies and fees, in order to ensure that the RQOs are met. Thirdly, **Managing water resource demands** - this is in order to keep utilisation within the limits required for protection. Lastly, **Continual monitoring**, involves monitoring the status of the country's water resources on a continual basis, in order to ensure that the RQOs are being met, and to enable DWAF to modify programmes for resource management and impact control as and when necessary.

The need for water resource protection is plain in light of the goods and services aquatic ecosystems provide, coupled with their threats of overuse. Water resource managers have needed clearly defined goals to facilitate the implementation of management objectives. World-wide this has prompted the development of different classification systems which identify different levels of protection according to kinds of use. The Australian and New Zealand Guidelines (ANZG) for Fresh and Marine Water Quality advocate 'Ecologically Sustainable Development' (ESD) principles, which imply 'acceptance of a degree of environmental degradation, as long as the integrity of ecosystems is not threatened' (ANZG, 2000). Guideline values are derived to protect 95% of aquatic life species but with varying levels of certainty that ecosystems will be protected. The required level of certainty decreases according to the degree of accepted modification of the natural system. Similarly, the Canadian Water Quality guidelines (CWQG) recommend benchmark values for the protection of 100% of species, 100% of the time but site-specific water quality objectives (WQOs) are adopted as is necessary for local conditions (CWQG, 2003).

In South Africa the National Water Act focuses on water resource protection to ensure efficient **use** and exactly how much use is permissible for a particular river or section of river is governed by a classification system. The system firstly classifies the current level of ecosystem health into one of five ecological Reserve categories (figure 1.1). Secondly, one of four water management classes is then selected defining the overall state towards which the water resource needs to be managed and an appropriate management class is assigned to the resource. The management objective, stipulated by the management class, is set according to the desired level of ecosystem health, and the associated level of resource protection. For example, a management objective exhibiting a high level of ecosystem health can be assigned a management class of 'A', associated with high levels of protection and restricted use of the resource. Only three

management classes exist as a 'poor' management class is not considered as a management option.

Excellent	Good		Fair	Poor	Management Classes and/or Ecological Categories
A	**B**	**C**	**D**	**E & F**	
Minimal	Slight	Moderate	Heavy	Unacceptable	**User impact**
Unmodified	Slightly modified	Moderately modified	Considerably modified	Critically modified	**Ecological condition**

Figure 1.1: South African proposed[1] ecosystem health and management classification system (Palmer et al., in press b)

Once a particular resource is classified, RQOs are determined. These are qualitative and quantitative targets or objectives set for each water resource in terms of the level of protection the water resource requires. The setting of RQOs is part of the Reserve determination process and serves to meet the requirements of the Reserve, i.e. both the basic human needs Reserve and the ecological Reserve. The ecological components of RQOs are derived using primarily Instream Flow Requirement (IFR) methods. The IFR can be defined as "that which describes, in space and time, the minimum amount of water that is felt will facilitate maintenance of the river at some pre-defined state" (King and Louw, 1998). Methods to derive IFRs include the Building Block Methodology (BBM) (King and Louw, 1998), Downstream Response to Imposed Flow Transformations (DRIFT) (Brown and King, 2000) and the Flow Stressor-Response (FS-R) method (O'Keeffe *et al*., 2002). The BBM rapidly provides scientific guidance on required flows for a river in cases where biological data and understanding of the functioning of the river are limited (King and Louw, 1998). The initial data-collection steps of the BBM closely approximate those of DRIFT and like BBM, DRIFT uses a holistic, scenario-based approach addressing all biophysical aspects of a river in question. A database is created which can be queried to describe biophysical consequences of any number of potential future flow regimes (scenarios). It is also designed to detail and quantify links between changing river condition and the social and economic impacts for the riparian communities who rely on the river for

[1] The classification system is still being refined by the Department of Water Affairs and Forestry (Palmer *et al*., in press b).

subsistence. Where the BBM 'builds up' a recommended flow regime from scratch, DRIFT takes the present day flow regime as a starting point. It describes the consequences for all aspects of the river when reducing or increasing flow regimes in different ways and it also addresses socio-economic links (Brown and King, 2000). The Flow Stressor-Response method is designed to be used within the BBM and DRIFT methodologies as a tool to guide the evaluation of the ecological consequences of modified low flow regimes, based on principles of Ecological Risk Analysis (Suter, 1993, cited in O'Keeffe and Hughes, in press). It aims to consistently capture specialist knowledge on the relationship between flow, hydraulic habitat and the responses of instream biota" (O'Keeffe *et al*., 2002). These relationships are then translated directly into stress profiles for any flow regime in terms of magnitude, frequency and duration. The method concentrates on water quantity requirements and is independent of the level of biological knowledge available however, this will affect the level of confidence associated with the recommended flows (O'Keeffe and Hughes, in press).

The concept of setting an IFR for any particular river complements the water resource objectives stipulated in the National Water Act of 1998. These include seeking out a balance between the need to protect and sustain water resources on the one hand, and the need to develop and use resources on the other. IFR methods link sound data to relevant management issues to ensure successful implementation. However, the IFR methods are limiting in that they deal only with flows and a water quality component to a very limited degree. Malan and Day (2002) outline how characteristic flows of rivers are intimately linked with water quality, because if flow is altered, water quality frequently changes. A recent approach has been outlined as part of a Reserve determination, termed the 'environmental water quality' (EWQ) approach and involves understanding how chemical, microbiological, radiological and physical characteristics of water link to the responses of living organisms and ecosystem processes (Palmer *et al*., in press a; Palmer *et al*., 2004d). In 1999, the Olifants River ecological Reserve determination was the first to include a comprehensive assessment of water quality (DWAF, 2000b). Since then methods for including water quality in Reserve determinations have been developed and refined, the most recent records of these are outlined in Palmer *et al*. (in press a).

Guidelines provide protective objectives as well as the structure needed by water resource managers in order to manage water quality. In doing so, they can support management decisions that are socially, economically and ecologically sustainable. They have also been an important component of water resource management over the

years (Hart *et al*., 1999). In South Africa, the South African Water Quality Guidelines for Aquatic Ecosystems were formulated in 1996 (DWAF, 1996a) and offered decision support for water resource managers (mainly the Department of Water Affairs and Forestry) in the management and protection of aquatic ecosystems (Dallas *et al*., 1998). Furthermore, the guidelines served as the primary source of information for determining the water quality requirements of different water users (DWAF, 1996c-h). However, the aquatic ecosystem guidelines were drafted prior to the establishment of a classification system aimed at protecting resources in an excellent or near 'natural' condition. Additional guidelines have subsequently been drafted which provide 'boundary' or 'trigger' values (discussion to follow) which are the numerical values or concentrations of a toxicant which allow managers to distinguish between the different ecological Reserve categories (Palmer *et al*., in press a).

One particular water quality constituent that has come under increasing surveillance over the last 15 years, and which is the main focus for this study, is salinity. Furthermore, salinisation has been identified as one of the major contributors to decreased water quality in South Africa (O'Keeffe *et al*., 1992) and warrants careful research and investigation.

1.1 Salinity and salinisation

One of the water quality parameters described in the South African Water Quality Guidelines for Aquatic Ecosystems is salinity or total dissolved salts/solids (TDS) (DWAF, 1996a).

TDS concentration is the total amount of material dissolved in a water sample and is measured in mg/L (Davies and Day, 1998b). Electrical conductivity (EC) is the measure of dissolved compounds in water that carry and electrical charge and is measured in mS/m (DWAF, 1996a). Salinity can be defined as the mass measurement of dissolved salts in a solution of given mass. Salinity is not exactly equivalent to TDS, however the two are closely related (US Dept of the Interior, 1999) (TDS concentration is directly proportional to EC). A common approximation for TDS concentrations from EC for South African inland waters is (DWAF, 1996a):

- TDS (mg/L) = EC (mS/m at 25 °C) x 6.5

The factor of 6.5 is approximate and can be influenced by the actual ionic make-up of the dissolved solids. It can be as high as 9.0 or as low as 4.0, however the 6.5 is fairly accurate for the average mixed ionic make-up of natural water (Urban-Econ, 2000b)

TDS can vary in natural waters according to geological formations and physical processes (e.g. evaporation), hence TDS is governed by constituent inorganic salts (DWAF, 1996a). Most commonly, the relative concentrations of major ions tends to be:

- Cations: $Na^+ > Ca^{2+}/Mg^{2+} > K^+$
- Anions: $Cl^- > HCO_3^- > SO_4^{2-} > CO_3^{2-}$

Salinisation is the process which refers to an increased concentration in water, or in soil, of naturally occurring mineral ions, particularly those of sodium (Na^+), chloride (Cl^-) and sulphate (SO_4^{2-}) (Davies & Day, 1998b). Salinisation is a useful measure of monitoring water quality because for the most part, the usefulness of water for most purposes diminishes with an increasing salt content (du Plessis and van Veelen, 1991). The process of salinisation can be driven by both natural and anthropogenic activities.

1.1.1 Natural Drivers of salinisation

Climate

The rate at which salinisation occurs is affected by various environmental components, such as annual rainfall, the ratio of precipitation to evaporation, groundwater hydrology and surface run-off rates. Seasonal and inter-annual variations in climate are also major drivers of solute concentrations in rivers (Interlandi and Crockett, 2003). The association of arid and semi-arid areas with high rates of salinisation is a common phenomenon (Kotb *et al*., 2000; Farber *et al*., 2004; Jorenush and Sepaskhah, 2003; Oren *et al*., 2004; Roos & Pieterse, 1995; Shanyengana and Sanderson, in press; Young, 2001), especially where these regions are associated with shallow, saline water tables (Jorenush and Sepaskhah, 2003). South Africa is typically characterised by large semi-arid areas. The average rainfall for the country is 450mm per year, well below the world average of about 860mm per year (DWAF, 2002). Its climate and landscape exacerbate the process of salinisation due to high evaporation:precipation ratios and low run-off:rainfall ratios. For South Africa, the runoff coefficient is only 10 per cent, meaning that 90 per cent of rainfall is lost through evapotranspiration. Evapotranspiration is defined as the combined water loss by evaporation from open water surfaces and from soil and groundwater through transpiration by plants (Rowntree, 2000). Some of South Africa's rivers, large dams, canals and farm dams suffer salinity problems due to evaporative losses from surface waters (1986a). Many

of the country's salinity problems have also occurred during, or as a result of, extreme climatic events.

Geology

Salinisation is often affected by the nature of geological formations of a particular area (Davies & Day, 1998b). The mass and type of mineral dissolved in solution depends on geochemical characteristics of the soil and the surrounding environment. Limestone bedrock and other calcareous sedimentary deposits can contribute to increasing solutes (Interlandi and Crockett, 2003). Decomposed shales release high concentrations of Na^+, Cl^- and SO_4^{2-}, while decomposed dolomites contribute principally calcium, magnesium and carbonate ions (Loewenthal, 1995). The mixing of leachates with formation water varies dependant on hydrological conditions (Farber *et al*., 2004). In the Jordan Valley, Jordan, sulphate-rich groundwater is derived from leaching of the Pleistocene and Neogene sediments (Pleistocene sediments induce dissolution of saline marls and gypsum) (Farber *et al*., 2004). In Namibia, groundwater found mainly in sedimentary terrain along ephemeral river flood plains displays seasonal salinisation (Shanyengana and Sanderson, in press). In South Africa, many water bodies are naturally high in dissolved salts, especially where rivers flow over old marine sediments such as the Karoo series (O'Keeffe *et al*., 1992). Dominance of specific ions is correlated with geographical patterns (Day and King, 1995). For example, ground waters of much of the country's coastal belt, and all of the Karoo, were categorised as 'highly mineralized chloride-sulphate waters with TDS values >1000mg/L (Day and King, 1995).

1.1.2 Anthropogenic drivers of salinisation

The process of salinisation can be exacerbated by anthropogenic activity, leading to unnaturally high levels of salinity in the natural environment. This is sometimes referred to as 'secondary salinisation' (Hart *et al*., 1991). The three main causes for increased salinisation due to anthropogenic activity include urban activity; industrial and mining operations, and agricultural activity and practices. In South Africa, urbanisation, industrialisation and irrigation have caused increases in salinity which greatly threaten the potential usefulness of the country's rivers (O'Keeffe *et al*., 1992).

Urban activity

World-wide, urban sprawl has resulted in an increase in dissolved solutes in rivers. In urban areas with high populations, high rates of freshwater abstraction exacerbate salinisation. This is especially so when water abstraction occurs during low flow periods, increasing salt concentrations in surface water (Young and Hillman, 2001). A United States example in the southeastern Pennsylvania region reveals a directly proportional relationship between increasing solute levels over the past several decades with quantity of developed land area in suburban portions of the Schuylkill catchment (Interlandi and Crockett, 2003). One of the main drivers in Northern hemisphere examples of salinisation is the use of road salts for de-icing (Interlandi and Crockett, 2003). Similar relationships between developed areas and solute levels have been observed in South Africa, for example in the Vaal River (Urban-Econ, 2000b). In the middle reaches of the Buffalo River, ecological integrity has been lost (due to salinisation and pollution), to such an extent that it has become a potential health hazard (O'Keeffe *et al*., 1992). With increased economic pressure, industrialisation also increases. This is likely to affect on salt levels in rivers throughout the country (Urban-Econ, 2000b).

Industrial and mining operations in relation to salinity

The mining sector within South Africa is diverse and water usage patterns and the impacts of increased salinity vary significantly throughout (Urban Econ, 2000a). Saline pollution by mineral salts, particularly those derived from irrigation seepages, mining and industrial effluents, and storm runoff from mining areas, has been documented as creating serious problems from as early as the 1970's in the Commission of Enquiry into Water Matters, Report of 1970 (DWAF, 1986a), contributing significantly to the country's salinity problems. One of the problematic factors of mining effluent is the contribution of acid mine drainage and sulphate pollution. In the process of coal mining in South Africa, coal deposits contain pyretic formations which, under certain conditions are oxidised to sulphuric acid and iron sulphate (Thompson, 1980). Resultant acid mine drainage from these by-products are extremely acidic and can be treated with hydrated lime ($CaCO_4$) before discharge into the environment. The resultant effluent is saline (gypsiferous) water, mainly due to Ca^{2+} and SO_2^{2-} in solution (Jovanovic *et al*., 1998). Another potential contributor to sulphate-enriched effluent is in the process of heavy mineral extraction from dune sand. The chemical impacts relating to smelting processes are of environmental concern. Effluent resulting from the smelter complex

are most likely to cause raised salinity levels in the receiving aquatic ecosystems, particularly due to the contribution of SO_4^{2-} ions (Palmer and Wade, 1997).

Examples of South African rivers that have been subject to intense pressure from mining activities include the Olifants and upper Vaal River catchments (Van der Merwe and Grobler, 1990). The Olifants River catchment formed the basis of one of the first comprehensive ecological Reserve determinations carried out in the country. The assessment revealed that various segments of the river are highly impacted by numerous coal mining and power generation activities and discharges from slime dams; not excluding the contribution of irrigation return flows and poor land use practices to increased salt loads in the system (DWAF, 2000b).

Agriculture

Agricultural activity can contribute to salinisation on a large geographical scale (El-Ashray *et al*., 1985). The main contributing factor to salt loading is irrigation, especially when saline groundwater is the significant or sole source of water (Oren *et al*., 2004). This results in the recycling of salts (mainly Cl^-, SO_4^{2-}, Na^+ and Ca^{2+}) dissolved in irrigation water. When water containing salts in solution is lost by evaporation and transportation, salts precipitate out, causing salt concentrations to increase. Major salts are not taken up substantially by plants and return to rivers and groundwater from water runoff from fields and by percolating through soil respectively. The problem intensifies as repeated irrigation results in increasing salt accumulation (El-Ashray *et al*., 1985). High rates of evapotranspiration and the lack of flushing by rain of near-surface and soil root zones in arid and semi-arid areas only exacerbate the process, increasing salt concentrations. Furthermore, infiltrating water from agricultural fields can cause water table levels to rise, increasing chances of evaporation (Kotb *et al*., 2000). In a study conducted in the Arava Valley in Israel, it was found that the main source of Cl^- in rivers came from irrigation water after evapotranspiration. Nitrate concentrations of recharging water are also easily affected as nitrate salts are highly soluble and easily flushed towards the water table (Oren *et al*., 2004).

Various factors have resulted in agriculturally-induced salinisation becoming a major problem in aquatic ecosystems worldwide. These include reduced annual flows, over-irrigation and insufficient drainage systems, over-use of salt-generating agrochemicals, the dumping of diverted saline springs or wastewater into freshwater systems, the intrusion of seawater into freshwater systems, and the accumulation of surface runoffs

in low-lying areas (Kotb *et al*., 2000). Most of these factors are linked either to insufficient planning, in the case of poorly designed irrigated systems, or are due to inadequate catchment management, in the case of poor land-use practices (e.g. land clearing) and overuse of natural resources within a catchment. Various countries have experienced problems in managing saline water bodies either caused by anthropogenic-related activities or due to rising saline groundwater tables and expanding saline lakes (Kefford, 1999; Orlob and Ghorbanzadehn, 1981). It is even thought that irrigated agriculture caused the decline of certain ancient civilizations (El-Ashry, *et al.* 1985).

In summary, contributing factors to salinisation in South Africa, as a result of agricultural activity, include runoff from dryland agriculture and irrigation return flows, including seepage losses from irrigation water storage and distribution systems, and flow reduction in rivers due either to upstream diversion of dilution waters or drought conditions (South African Water Bulletin, 1990).

As previously discussed, South Africa has large semi-arid areas, which implies that it shares some of the above-mentioned problems of salinisation associated with agricultural activity. However, the potentially negative affects of salinisation on South Africa's natural environmental is not well-documented and it was only in 1986 that the Department of Water Affairs clearly identified salinisation as one of the main water quality problems experienced in the country, alongside with eutrophication (DWAF, 1986b).

1.1.3 Effects of salinity on aquatic ecosystems

The focus of the salinity problem in South Africa has centred mainly on mining and agricultural activities, and early emphases were placed on researching de-salinisation processes to help meet supply demands of industry, agriculture and urban centres (DWAF, 1986a). Limited focus was given to the salinity problem in terms of management of the country's aquatic resources. In small quantities, salt is essential for life; however, when it reaches greater concentrations in the natural environment it has the capacity to adversely affect biota (Beresford *et al*., 2001). Some isolated references in the literature do examine the effects of salinity on aquatic ecosystems, however it is only recently that salts have been formally recognised as toxicants (Kefford *et al*., 2002). The big question that arises in the salinity debate is: 'what impact, positive or

negative, does an increase of salinity (either naturally- or anthropogenically-induced) have on aquatic ecosystems?'

Some fauna and flora are able to cope with varying degrees of salinity. Usually these organisms' coping mechanisms have a threshold beyond which, variable salinity conditions can directly and indirectly affect ecosystem structure and function. The main concern for aquatic organisms associated with salinity is osmotic stress, when cells of the organisms have either a lack of water or an excess of ions (or both) that can result in a range of toxic effects (Hart *et al*., 1991). Most aquatic organisms are stenohaline, (can only adapt to a narrow range of salinities) hence salinity changes might affect aquatic organisms in two ways (ANZG, 2000):

i) direct toxicity through physiological changes (particularly osmoregulation) – both increases and decreases in salinity can have adverse effects;
ii) indirectly by modifying the species composition of the ecosystem and affecting species that provide food or refuge.

Hart *et al*. (1991) provides a comprehensive review on the salt sensitivity of Australian freshwater biota which is useful in understanding how unnatural levels of salinity can affect biota. On a fundamental level, salinity increases can affect microbe communities at three levels: effects and adaptations of individual types; effects on community structure; and effects on microbial processes, such as metabolism and nutrient recycling (Urban-Econ, 200b). Algae can be indirectly affected when turbidity levels lower due to a change in levels of TDS in the water. This results in an increase of light penetration as colloids precipitate out (Urban-Econ, 2000b).

In terms of flora, riparian vegetation species that are most vulnerable to salinity increases are 'non-halophytes', which achieve best growth conditions in non-saline conditions and whose growth is reduced as salinity increases. Such species compose the majority of riparian flora of streams and associated wetlands (Hart *et al*., 1991). Growth reduction occurs in these plants when, in highly saline environments, ion uptake reaches toxic levels, or the plant diverts it's metabolites to maintenance respiration. A further consideration for plants in salt-affected land is prolonged or occasional waterlogging. Recent studies suggest waterlogging and increasing salinity can act synergistically. Salinity appears to interfere with the plant's adaptations to waterlogging and vice versa. It also appears that tolerance to the combined effects of salinity and waterlogging are greater in waterlog-tolerant species (Hart *et al*., 1991).

Freshwater invertebrates can also be adversely affected by salinity as they are generally hyper-osmotic regulators (Hart *et al*., 1991). Hence, they are incapable of maintaining body fluid solute concentrations below that of the water they live in. If the salinity of their environment is increased above a threshold level, they take up increasingly more ions which results in increasing water loss from cells. In extreme circumstances the organism will no longer function properly and dies. For invertebrates, factors such as degree of acclimation, life stage and temperature can influence sensitivity to salinity increases. An organism's condition such as reproductive state, stage within a moult cycle, sex and body size and nutritional state can affect an invertebrate's ability to regulate cell solute concentrations in varying external salinities (Hart *et al*., 1991). Varying salinities can further modify community structures of macroinvertebrate communities (Kefford, 1998).

Fish are known to have coping mechanisms to deal with varying salinities in their surrounding environment, i.e. they use either hyper-osmotic (transport ions across gill surfaces) or hypo-osmotic regulation (loose water through gills) (Hart *et al*., 1991). A large proportion of fish species have marine origins and as a result have some inherent tolerance to salinity. In conditions where chemical conditions are variable, freshwater species may be more likely to retain characteristics of salinity tolerance. Life stages can also affect tolerance capabilities as larval fish do not have adult osmoregulatory abilities, placing them at higher risk in conditions of varying salinity (Hart *et al*., 1991. Further discussion pertaining to salt sensitivities of amphibians, mammals and birds can be found in literature (Hart *et al*., 1991; US Dept of the Interior, 1999).

On a more widespread scale, salinisation can threaten biodiversity hotspots in areas associated with high species diversity and with rare species of fauna and flora. The South-West region of Australia, has a pending 'salinity crisis', threatening the natural environment with potential large scale loss of species diversity (Beresford *et al*., 2001).

Worldwide, South Africa included, the health of aquatic ecosystems is being threatened by anthropogenically-induced salinisation. In order for these particular ecosystems to be managed accordingly to prevent further degradation and unsustainable use of the resource, the impact of specific salinity levels in the aquatic environment need to be quantified. Toxicology and ecotoxicology can be used as valuable tools to quantify the impact of salinity increases on aquatic ecosystems.

1.2 Ecotoxicology

Toxicology in the broader sense has been defined as that which 'is concerned with the deleterious effects of chemical and physical agents on all living systems' (Plaa, 1998). More specifically, environmental toxicology is a field of study that deals with 'the potentially deleterious impact of chemicals, present as pollutants of the environment, to living organisms (Plaa, 1998). Incorporated within the field of environmental toxicology is aquatic toxicology. This has been defined as '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 subcellular through individual organisms to communities and ecosystems' (Rand *et al*. 1995). Ecotoxicology is an area of study that has evolved as an extension of environmental toxicology as it is also 'concerned with the toxic effects of chemical and physical agents on living organisms', but especially 'in populations and communities within defined ecosystems' and is known to include the study of 'the transfer pathways of those agents and their interactions with the environment' (Plaa, 1998).

Ecotoxicology can facilitate the implementation of ecosystem protection by contributing to a risk-based approach, accommodating the unpredictability, uncertainty and complexity of aquatic ecosystems. Ecotoxicology can be used to quantify the impact of increasing salinity to biota. One way in which this can be done is by conducting acute toxicity tests using a salt as the toxicant and to generate tolerance data of different types of aquatic biota at various concentrations. Single-species acute toxicity tests have been preferred over multi-species bioassays and chronic toxicity testing. This is because single-species tests are relatively simple, easy to standardise and are reproducible and rapid, whereas multi-species bioassays are generally expensive, time-consuming and particularly, *in-situ* bioassays are characterised by high variability (ANZG, 2000). However, the shortcoming of single-species tests is that they exhibit low levels of biological and environmental realism, excluding significant inter-species and ecosystem-level interactions. Despite this, such data continue to dominate toxicity data sets and are mostly used in the derivation of water quality guidelines (WQGs) (Schudoma, 1994). In some cases acute to chronic extrapolations can also be used, but ideally acute toxicity data should be combined with data from comprehensive field data where possible when setting management objectives. These methods and also the philosophies surrounding the use of toxicity data are variable and have developed over the last two decades (Rand *et al*., 1995).

1.3 Use of toxicity data in the development of water quality guidelines

Ideally, WQGs should allow for the sustainable functioning of healthy and balanced aquatic ecosystems. The setting of WQGs or the use of water quality criteria has proven to be an important tool in supporting resource management decisions (Roux *et al*., 1996). Various methods have been applied in using toxicity data to set numerical data for specific substances that indicate levels at which protection of sensitive components of aquatic ecosystems can be ensured (Roux *et al*., 1996).

One of the earliest approaches to guideline development was the use of the assessment factor (AF) approach. In this approach, the lowest reported toxicity value is divided by a constant that is variously called an assessment, uncertainty, application or safety factor. The magnitude of the AF is governed by the perceived 'quality' of the toxicity data (Warne, 1998). Assessment factor methods have been used in the United States and Australia by the United Stated Environmental Protection Agency (USEPA, 1994) and the Australian and New Zealand Environment and Conservation Council (ANZECC, 1992), respectively. They have also been used in South Africa based largely on the methodology developed by Stephan *et al*. (1985). However, more recent approaches have shifted towards the use of statistically based extrapolation methods (Warne, 1998). The stimulus for extrapolation methods has been due firstly to the need for a risk-based approach, secondly to a lack of existing multiple species experiments and thirdly, to the lack of scientific basis for assessment factors.

Extrapolation methods use toxicity data obtained from tests on individual species and fit a statistical distribution to the data to derive a concentration that should protect 95% of the species in the environment (Warne, 1998). Extrapolation methods have been adopted in the Netherlands, Denmark as well as the United States and are best explained according to three main types of extrapolation techniques, grouped according to the authors that have described them:

1. Stephan *et al*., 1985.
2. Wagner and Løkke, 1991.
3. Aldenberg and Slob, 1993 (developed from the methodology first proposed by Kooijman, 1987 and Van Straalen and Denneman, 1989).

The technique proposed by Stephan *et al*. (1985) has been rejected for several reasons (Warne, 1998):

1. It assumes a threshold toxicity value below which no detrimental effects will occur. This is not supported by scientific literature and risk assessment theory.
2. It assumes ecosystems can tolerate high concentrations for short periods of time.
3. It has extensive data requirements.

As proposed by Warne (1998) the Aldenberg and Slob method (1993) is the most favourable technique, over the Wagner and Løkke (1991) method. This is because it is recommended by the Organisation for Economic and Co-operation and Development (OECD); is adopted in the Netherlands; and has received more validation work than the Wagner and Løkke (1991) method.

Hence, this research aims to follow international trends by adopting the Aldenberg and Slob (1993) method in the process of developing water-quality criteria, by using species sensitivity distributions (Chapter 3), in contrast to the original assessment-factor approached proposed by Roux *et al*. (1996).

Although this study focuses on the toxicity of two specific salts (NaCl and Na_2SO_4), previously attention has focused on the differential toxicity of different salts and their ionic compositions once in solution. Mount *et al*. (1997) acknowledges the need for a broader understanding of major ion toxicity and that salinity toxicity is dependant on ionic composition. In the South African context, Jooste and Rossouw (2002) considered the varying toxicity of ionic compositions when proposing benchmark or boundary values for the boundary between the Natural/Good and Fair/Poor ecological Reserve categories (figure 1.1). Jooste and Rossouw (2002) developed a model for Toxicologically Important Major Salts (TIMS) which takes into consideration ionic ratios and concentrations, and derives both a Lethality Benchmark and a Sub-Lethality Benchmark. Acute and Chronic endpoints from the USEPA AQUIRE (USEPA, 2002) database are extrapolated to 336 hours (time at which toxicity reaches a steady state) to derive the lethality and sub-lethality benchmarks respectively. This study adopts the idea of using acute toxicity data in deriving the salinity boundary values for the Fair/Poor ecological Reserve categories but uses a Species Sensitivity Distribution (SSD) approach instead of a direct data extrapolation approach. It also focuses on two main salts, sodium chloride and sodium sulphate, rather than ionic complexes.

An important component in deriving SSDs is having large sets of data with which to work. The greater the amount of data used in SSD derivation and the broader the taxonomic representation of that data, the more accurately the SSD is likely to

represent 95% of the species in the ecosystem. Thus, toxicological databases serve as important tools in accurately deriving WQGs.

1.4 Toxicological databases

Various methods have been developed and are in the process of being developed to use toxicity data in deriving WQGs. One of the most practical ways to access toxicity data is through toxicological databases. A prominent international toxicity database currently used is the AQUIRE database, which was created and is currently maintained by the USEPA, the Office of Research and Development (ORD) and the National Health and Environmental Effects Research Laboratory's (NHEERL's) Mid-Continent Ecology Division (USEPA, 2002). The database provides single chemical toxicity data for aquatic life and terrestrial plants as well as wildlife. The original intended use for this database was for accessing toxicity information for use in characterizing, diagnosing and predicting effects associated with chemical stressors and to support the development of 'ecocriteria' for natural resource use (USEPA, 2002). This database has been used extensively in the development of WQGs for South Africa because indigenous organism response data specific to South Africa are limited (DWAF, 1996a; Roux *et al*., 1996) and no standard, official database of toxicity data currently exists in the country.

In an effort to reduce this dominance of internationally-based data, the Unilever Centre for Environmental Water Quality (UCEWQ) as part of the Institute for Water Research (IWR) (Rhodes University, Grahamstown) has a toxicological database of South African aquatic macroinvertebrate tolerances. Data generated from this study has contributed directly to this database (Palmer *et al*., in press b). Ideally this database will expand in the future, incorporating data from a wider variety of toxicants, across a broader range of taxonomic groups, so as to increase the accuracy and representivity of toxicity data used in the ongoing development of South African Water Quality Guidelines.

1.5 Motivations and Aims

The above discussion has served to highlight several motivations for the aims of this study. These **motivations** are:

1. South Africa's current WQGs were formulated using mainly international data. It is important to include South African species information in the development of guidelines, specifically for salts, as salt combinations and exposures are geographically distinct.
2. Unnatural increases in salinity are evident in certain regions of South Africa's rivers. These high levels of unnaturally occurring salinity levels can potentially have adverse affects on aquatic ecosystems and their biota.
3. Ecotoxicology is a useful tool to develop risk-based WQGs and WQOs, which can then be applied by water resource managers, with a relevant level of confidence, in the process of decision making.
4. Macroinvertebrates are recommended test organisms in toxicity tests, however, test protocols using species indigenous to South Africa are lacking.
5. Current South African toxicological databases for response data for indigenous organisms to salinity need expansion.

Hence, the aims of this study are to:

1. Generate acute toxicity data for salinity so as to expand the acute salinity toxicity database and thereafter use a wide range of freshwater invertebrate taxa to assess the relevance of Species Sensitivity Distribution-based salinity guidelines.
2. Evaluate the role of acute toxicity data in the development of salinity guidelines and make recommendations to water quality managers and those responsible for deriving WQGs.

CHAPTER 2: A comparison of acute salinity tolerances of selected South African freshwater macroinvertebrates

2.1 Introduction

In this chapter the foundation is laid for the use of acute (lethal) toxicity data in the derivation of species sensitivity distributions (SSDs) for NaCl and Na_2SO_4. A discussion of the history and theoretical background of SSDs follows in chapter 3. The use of SSDs was proposed by Aldenberg and Slob (1993) and essentially involves data extrapolation. It can be used to derive protective concentrations (PC) at any theoretical level of species, for example 95% of the species in an ecosystem. A SSD uses tolerance data derived from toxicity tests to derive a PC. The PC is used as the water quality guideline and is referred to as the trigger value (TV) or boundary value (BV). In order to ensure that the derived PC is representative of the ecosystem it is protecting, the toxicity data used to derive the SSD should include tolerances of indigenous organisms from as wide a range of taxa as possible (Newman, *et al*., 2000).

Protocols for standard toxicity tests in South Africa are based on internationally accepted protocols that are dominated by non-indigenous test organisms such as daphnids (e.g. *Daphnia magna*), guppies (*Poecillia reticulata*) and fathead minnows (*Pimephales promelas*) (Cooney, 1995). As a result, few protocols exist for using indigenous South African species in toxicity tests (DWAF, 2000a) and hence, South African water quality guidelines are currently heavily weighted by international toxicological response data.

In this study wild-caught individuals from populations of indigenous macroinvertebrates were used in acute toxicity tests, using inorganic salts as toxicants, to generate acute salinity response data representative of South African aquatic ecosystems and their species. Aquatic macroinvertebrates make good test organisms as they are widely used as indicators of biodiversity and river health (Davies and Day, 1998c, Kefford *et al*., 2003b). Only acute 96 hour tests were conducted due to the time consuming and complex nature of chronic toxicity tests and the need to test as many taxa as possible. Many of the organisms used in this study had not previously been used in toxicity tests. Complicating factors such as testing predacious organisms and organisms collected

from lentic aquatic systems, necessitated a deviation from the existing protocol. Various designs in experimental chambers were developed to accommodate these factors.

It is unrealistic to conduct tests for all salts, hence this study uses a simplified model to conduct acute toxicity tests using sodium chloride and sodium sulphate. Two of the most common cations and anions in South Africa's surface waters are Na^+ and Cl^- are respectively (Day and King, 1995) and their concentrations are commonly exacerbated through agricultural activities. The SO_4^- ion is known to exacerbate salt toxicity (Jooste and Rossouw, 2002) and is a common ion associated with mining effluent (Thompson, 1980; Jovanovic *et al*., 1998).

Acute toxicity data generated in this study are placed in the context of international data using SSDs and comparisons drawn between salinity responses of South African organisms, mostly invertebrates, and other non-indigenous organisms.

This chapter aims to:

1. Report on toxicity data generated for species of indigenous freshwater macroinvertebrates not previously used in toxicity tests.
2. Make recommendations for future work using indigenous freshwater macroinvertebrates.
3. Provide data for use in deriving SSDs relevant to South Africa by generating tolerance data using indigenous freshwater macroinvertebrates in 96 hour acute toxicity tests. These SSDs can then be used to formulate relevant WQGs for 2 inorganic salts, specific to South African freshwater ecosystems (chapter 3).

2.2 Study area and methods

2.2.1 Study sites and organism collection

Wild-caught indigenous macroinvertebrates were selected as test organisms (DWAF, 2000a). Fourteen species were collected from four study sites in the Eastern Cape Province, South Africa (figures 2.1 and 2.2). Study sites were relatively unimpacted to ensure that organisms were not previously exposed to deteriorating water quality conditions (DWAF, 2000a). The locations of the four study sites, and the species collected at each site, are summarized in table 2.1. The first site, characterized by

riffles and situated in the Kat River (figures 2.2 and 2.3), is in close proximity to Amhurst Village. The Kat River has its source in the Hogsback Mountains, flows through the small town of Fort Beaufort and eventually joins the Great Fish River (figure 2.2). The second site, also characterised by riffles, is situated in the Balfour River (figures 2.2 and 2.4), which is a tributary of the Kat River.

Figure 2.1: ***Map of the provinces of South Africa showing the location of the Eastern Cape***

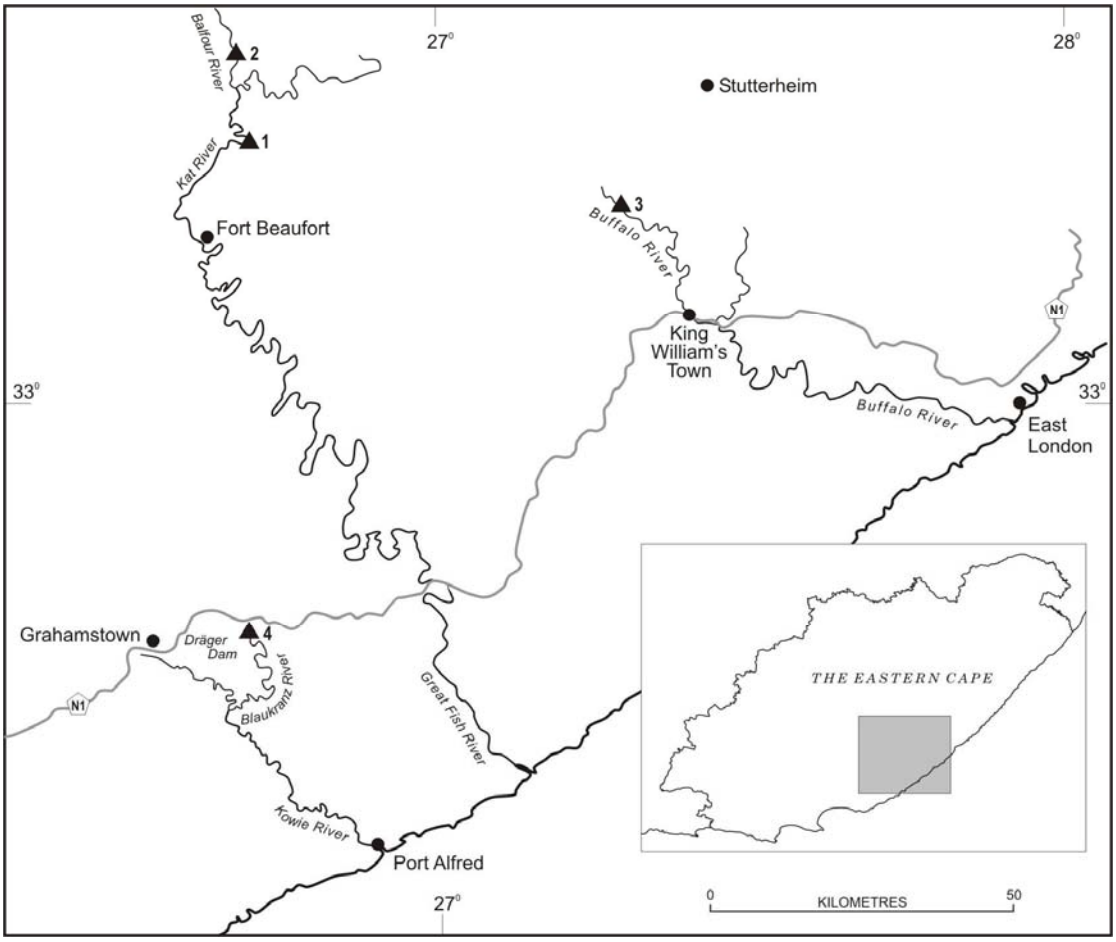


Figure 2.2: ***Map showing location of all four study sites (Site 1 – Kat River, Site 2 – Balfour River, Site 3 – Buffalo River and Site 4 – Dräger Dam)***

Table 2.1: ***Summary of collection sites, habitat descriptions and species collected at each site***

Site no.	Site name	GIS co-ordinates	Site description	Species collected	Common name
1	Kat River	32°38'30"S, 26°41'15"E	Riffles	*Afronurus barnardi* (Heptageniidae)	Mayfly
				Euthraulus elegans (Leptophlebiidae)	Mayfly
				Tricorythus discolor (Tricorythiidae)	Mayfly
				Burnupia stenochorias (Ancylidae)	Freshwater limpet
2	Balfour River	32°31'45"S, 26°40'50"E	Riffles	*Baetis harrisoni* (Baetidae)	Mayfly
				Demoreptus natalensis (Baetidae)	Mayfly
				Oligoneuropsis lawrencei (Oligoneuriidae)	Mayfly
3	Buffalo River	32°43′55"S, 27°17'50"′E	Riffles	*Caenid* sp.1 (Caenidae)	Mayfly
				Simulium medusaeforme (Simulidae)	Black fly
				Simulium sp. (Simulidae)	Black fly
				Suragina sp. (Athericidae)	Snipe fly
4	Dräger Dam	33°18'15"S, 26°40'45"E	Lentic system	*Cloeon virgilae* (Baetidae)	Mayfly
				Plea pullula (Hemiptera)	True bug
				Ischnura senegalensis (Coenagrionidae)	Damselfly
				Enallagma sp. (Coenagrionidae)	Damselfly

Figure 2.3: Kat River sampling site in close proximity to Amhurst Village

Figure 2.4: Balfour River sampling site

The third collection site, also a riffle, was situated above Maden Dam along the Buffalo River (figures 2.2 and 2.5).

Figure 2.5: Buffalo River sampling site

Mayfly larvae from the Kat, Balfour and Buffalo Rivers were collected according to methods recommended by DWAF (2000) except for Oligoneuriidae larvae from the Balfour River which were collected by dislodging larvae into a hand-held net by rubbing a hand gently over an area of bedrock positioned in fast flowing water. Larvae were transferred to cooler boxes containing river water and were equipped with battery-operated air pumps to maintain high oxygen levels during transportation (DWAF, 2000a). Limpets were collected and transported according to methods described by Davies-Coleman (2002). Athericid larvae were collected using a modification of the SASS collection method for stones in current (Davies and Day, 1998c) (collection time was not limited). Dislodged debris and organisms collected in the SASS net were transferred to a SASS tray and athericids sorted into 5 litre, aerated plastic jugs, aerated, before being transferred to an aerated cooler box containing river water for transportation. Simulidae were collected from riffle rocks by gently removing larvae using paintbrushes. Between five and ten larvae were placed in plastic petri dishes lined with moistened filter paper, placed on a layer of crushed ice (De Moor, 2002) in a large plastic container, for transportation.

Lentic organisms were collected from a fourth study site, Dräger's Dam situated on a private farm, 15km outside of Grahamstown (figures 2.2 and 2.6).

Figure 2.6: Dräger Dam sampling site

The collection site, a lentic system characterised by marginal vegetation, is one of three catchment dams. The first of the dams fills up with surface runoff from the surrounding catchment during rainfall events which overflows into the second dam, which in turn overflows into the third dam. The second dam was chosen as a collection site due to easy accessibility and an abundance of marginal vegetation.

All organisms from the dam were collected using hand-held nets and transferred to plastic bags containing river water and marginal vegetation, supported in a large bucket for transportation. No aeration was deemed necessary, due to a short transportation time of 20 minutes. The following were recorded at all field sites: electric conductivity (EC) in mS/m, TDS in ppm, pH, temperature in °C and dissolved oxygen (DO) in mg/L.

2.2.2 Identification of test organisms

Species identification was confirmed by the curators of the National Freshwater Invertebrate collection at the Albany Museum (De Moor and Barber James, pers. comm.) with the exception of two species. *Burnupia stenochorias* was identified by Dr Heather Davies-Coleman of the Unilever Centre for Environmental Quality-Institute for Water Research (UCEWQ-IWR), Grahamstown. Athericid larvae were identified as a *Suragina sp.* by Dr Brian Stuckenberg of the Natal Museum. Attempts to rear Athericid larvae to adults at the UCEWQ-IWR failed. The current collection of Caenidae at the Albany Museum is described according to 10 different species. The Buffalo River

Caenid nymph was described as *Caenid* sp.1 (Barber-James, pers. comm., see appendix 1). Two species of Simulid larvae were identified: the first *Simulium medusaeforme* of the sub-genus *metomphalus* and the second, also a Simulium species of the sub-genus *Pomeroyellum* (identification of simulidae was not confirmed to species level as these data were excluded from final results due to unsuitable toxicity test results with this organism).

Coenagrionid larvae were reared through to adult stage to facilitate species identification. Coenagrionidae nymphs were reared by adapting a method described by Samways and Wilmot (2003). Modified honey jars were used as rearing vessels. Each honey jar had two squares cut from either side of it and this was replaced with a plastic mesh secured on with silicon. A hole was cut in the top of each lid. Each honey jar had two holes cut on either side near the top and threaded through with string so that the jars could be secured onto the sides of the glass tank. Six jars were then partly submerged into the glass tank filled with dechlorinated tap water. The tank was aerated with two aerators. A strip of plastic gauze was placed in each jar onto which the emerging adult could climb. One nymph of approximately 2cm in length was placed in each jar. The nymphs were fed with mosquito larvae (Samways and Wilmot, 2003) and small baetid nymphs. Five coenagrionid nymphs were reared successfully between days 13 and 34 after commencement of rearing. Reared specimens were sent to the Albany Museum, Grahamstown for identification. One species was identified as *Ischnura senegalensis* and the other identified to genus level, *Enallagma sp*.

Further investigation of the coenagrionid nymphs was conducted to determine diagnostic nymphal characteristics that could allow for the experimental sample to be separated out into the two sets of tolerance data for two different species. The distinguishing traits between the two species were the number of premental setae, but because early instar life stages were used, and the identification guides were formulated for identification at late instar nymphal stages (Samways and Wilmot, 2003), the test individuals could not be separated (premental setae could not be accurately counted as these were still developing at the instar stages). Hence, the coenagrionid larvae were treated as a genus complex comprising *Ischnura senegalensis* and *Enallagma* sp. and the number of organisms per experimental vessel was increased so as to minimise the chance of unequal species representation in each experimental concentration.

2.2.3 Experimental systems

Salt exposure experimental systems were set up prior to field collection of organisms. Artificial stream systems or channels (figure 2.7) were used for organisms collected from lotic environments (Balfour, Kat and Buffalo Rivers) (DWAF, 2000a). This protocol was adapted for the freshwater limpet *Burnupia stenochorias* (Ancylidae). Limpets were placed on plastic Petri-dishes and then submerged in the channel to allow the limpets an immediate and easy foothold on a substrate. Athericidae are known to be predacious and were therefore used in the lentic experimental system (50ml plastic vials, see below) to keep larvae separate from each other[1].

Using the underlying principles of toxicity testing described in DWAF (2000), an experimental system was designed to accommodate organisms collected from a lentic aquatic system, i.e. Dräger Dam. The system was designed for predacious damselflies (Coenagrionidae) which had to be accommodated individually to avoid cannabalism (Samway and Wilmot, 2003). The bottoms of 50ml plastic vials were sawed off, and a large hole was drilled in the lid (figure 2.8). The containers were then turned upside down and mesh gauze was secured in the bottom with the lid. Two further holes were drilled on opposite sides to allow several containers to be suspended on a single dowel stick, which was then suspended across the rim of a glass tank (figure 2.9) allowing three quarters of the container to be submerged in solution. Each tank was vigorously aerated to maintain oxygen levels in each vial over the experimental period. Glass tanks were replaced with plastic tubs (figure 2.10) to increase capacity of each experimental tank (from 24 to +40 vials) to allow for the variability introduced by two coenagrionid species. Vials were also used to conduct experiments using the *Cloeon virgilae* (Baetidae) collected from Dräger Dam with approximately five baetids allocated to one pill container during a toxicity test.

Similarly, glass tanks were used with larger 500ml plastic jars to provide leptoceridae and pleiidae with an area for swimming. Jars had two squares cut from both sides and a plastic mesh secured in their place, using silicon. The plastic mesh allowed for circulation. Two holes were drilled in the top of each jar. String was threaded through

[1] At the time of the experiment being conducted, no lotic experimental system had been developed to keep predacious organisms separate from each other. The static system appeared adequate as no control mortality was observed and tolerance data met the assumptions of the quality criteria used in the study.

these holes and, using the string, the jars were secured to a single dowel stick with three jars per dowel stick.

Figure 2.7: ***Artificial stream systems or channels used for organisms collected from lotic enviornments***

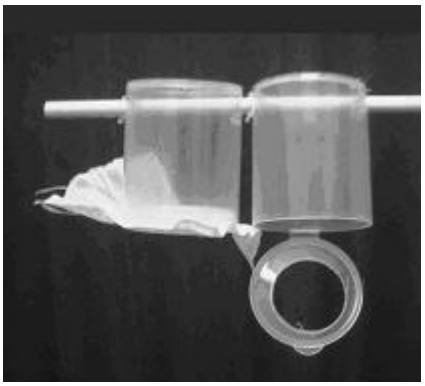


Figure 2.8: ***Two single 50ml plastic vials illustrating how gauze is secured into container***

Figure 2.9: ***Lentic experimental system – aerated glass tanks with individual 50ml plastic vials (suspended on wooden dowel sticks) to accommodate predacious organisms***

Figure 2.10: Lentic experimental system – aerated plastic tubs containing 40+ 50ml plastic vials suspended by string on wooden dowel sticks

2.2.4 96 hour acute toxicity tests

De-chlorinated tap water was used as the diluent medium (prepared by passing tap water through a carbon filter) (DWAF, 2000a). Experimental vessels were filled with de-chlorinated tap water and allowed to cool to laboratory temperature to ensure constant temperature of diluent medium. Each lentic experimental vessel was aerated using air stones connected to air pumps with plastic tubing. After transportation, organisms were transferred into experimental vessels excluding damaged organisms and those with wingbuds (indicating last instar before emergence) and allowed 36 hour acclimation time (DWAF, 2000a). Prior to toxicants being added, experimental vessels were examined and dead organisms removed, recorded as acclimation mortalities and preserved in 70% ethanol to confirm identification. Vessels exhibiting >10% acclimation mortality were rejected from the experiment (DWAF, 2000a). After acclimation, toxicant solutions (NaCl or Na_2SO_4) were made up at selected concentrations using de-chlorinated tap water as the experimental medium. Where estimated LC_{50}s for particular species were unknown, range finding tests were undertaken by selecting a wide range of concentrations to estimate the concentrations to be used for a definitive test. Details of number of organisms used per experimental vessel, number of experimental vessels used, concentrations used per experimental vessel and number

of controls used are provided for NaCl and Na_2SO_4 in tables 2.2a and 2.2b respectively.

Daily measurements of the following were taken in each experimental vessel, after organisms were exposed: electric conductivity (EC) and TDS using Amel 160 and Cyberscan 200 conductivity meters; pH using Cyberscan 10 and Beckman 10 pH meters; DO using WTW OXI92 dissolved oxygen meter and temperature using a thermometer (0-50°C). Immobility was used as a surrogate for death as the test endpoint (Cooney, 1995). Immobility was recorded at 12 hour intervals for a period of 96 hours. Immobile organisms were collected at each interval, labelled and preserved in 70% ethanol. Surviving organisms at the end of the 96 hour period were collected, labelled and preserved in 70% ethanol. Two water samples were collected from each experimental vessel at the end of the 96-hour period, one for macro-elements, nutrients, phosphate, ammonium and DO (each sample was preserved with mercury chloride) and the second for metal analysis. These were sent to the Department of Water Affairs and Forestry (Resource Quality Services-DWAF) in Pretoria for analysis. Laboratory equipment was washed and cleaned in detergent and 2% hydrochloric acid.

Table 2.2a: ***Summary of number of organisms used per experimental vessel, number of controls used, number of experimental vessels and concentrations used per experimental vessel, for experiments using NaCl***

Exp No.	Organisms	No. of organ-isms	No. of controls	No. of experimental vessels (excl. controls)	Concentrations (mg/L)
1	*Euthraulus elegans*	30			
	Oligoneuropsis lawrencei	35			
	Demoreptus natalensis	20			100; 500; 1000; 2000; 3000; 5000; 6000;
	Baetis harrisoni	30	1	9	8000; 10 000
2	Coenagrinidae	28	1	7	100; 1000; 2000; 5000; 10 000; 20 000; 40 000
3	*Cloeon virgilae*	50	3	8	500; 1500; 3000; 5000; 6000; 7000; 8000; 10 000
	Plea pullula	30	1	6	500; 1500; 5000; 6000; 8000; 10 000
4	Coenagrioid sp.	40	1	9	5000; 10 000; 15 000; 20 000; 23 000; 27 000; 30 000; 35 000; 45 000
5	*Leptoceris* sp.	45			100; 500; 1000; 5000; 10 000; 20 000; 40
	Plea pullula	30	1	7	000
6	*Leptocerid* sp.	45	1	9	500; 1000; 2000; 4000; 6000; 8000; 10 000; 15 000; 30 000
7	*Plea pullula*	45	1	10	100; 1000; 2000; 4000; 6000; 8000; 12 000; 15 000; 20 000; 40 000
8	Coenagrionidae	40	1	8	7000; 15 000; 20 000; 23 000; 27 000; 30 000; 35 000; 45 000
9	*Baetis harrisoni*	25			
	Burnupia stenochorias	25			
	Demoreptus natalensis	23	1	9	50; 100; 200; 280; 320; 350; 400; 500; 800
11	*Caenid* sp.1	10	1	5	500; 1000; 4000; 10 000; 20 000
	Simulidae	40	1		500; 1000; 4000; 7000; 10 000; 20 000; 40
	Suragina sp.	10	1	7	000

Table 2.2b: ***Summary of number of organisms used per experimental vessel, number of controls used, number of experimental vessels and concentrations used per experimental vessel, for experiments using Na_2SO_4***

Exp No.	Organisms	No. of organ-isms	No. of controls	No. of experimental vessels (excl. controls)	Concentrations (mg/L)
1	*Tricorythus discolor*	26			
	Afronurus barnardi	28			100; 1000; 3000; 4000; 5000; 6000; 7000;
	Burnupia stenochorias	10	1	9	8000; 12000
3	*Cloeon virgilae*	40	3	8	8000
	Plea pullula	30	1	6	100; 500; 1000; 2000; 3000; 4000
	Coenagrionidae	12	1	5	5000; 8000; 15 000; 25 000; 40 000
4	Coenagrinoid sp.	40	1	9	5000; 15 000; 20 000; 24 000; 28 000; 32 000; 35 000; 40 000; 45 000
5	*Leptoceris* sp.	45			100; 500; 1000; 5000; 10 000; 20 000; 40
	Plea pullula	30	1	7	000
6	*Leptoceris* sp.	45	1	9	500; 1000; 2000; 4000; 6000; 8000; 10 000; 15 000; 30 000
7	*Plea pullula*	45	1	10	100; 1000; 2000; 5000; 6000; 7000; 8000; 10 000; 15 000; 40 000
8	Coenagrionidae	40	1	8	10 000; 20 000; 24 000; 28 000; 32 000; 35 000; 40 000; 45 000
9	*Baetis harrisoni*	25			
	Burnupia stenochorias	25			50; 200; 300; 400; 500; 600; 700; 800; 10
	Euthraulus elegans	35	1	10	000; 12 000
10	*Demoreptus natalensis*	20			
	Oligoneuropsis lawrencei	30	1	8	50; 100; 300; 400; 500; 600; 800; 1200

2.2.5 Data analysis

Data screening

In order to confirm that nominal concentrations were equal to that of actual measured concentrations, nominal concentrations (in mg/L) were compared to measured ECs (in mS/m) using regression analysis conducted using STATISTICA statistical software package (StatSoft, Inc., 2003). Experiments exhibiting a p-value >0.05 were identified for further analysis due to high uncertainty associated with the relationship between nominal concentrations and measured ECs. Further analysis involved plotting daily EC readings against nominal concentrations using an XY scatterplot. Experiments yielding EC readings inconsistent with nominal concentrations were rejected.

Analysis of toxicity data

Nominal concentrations were used to calculate all LC_{50} values for use in the SSD analyses. The parametric, Probit method was used to calculate LC_{50} values where the experimental data fit the assumption of the method. In this method, a parametric

normalised distribution of the percentage of organisms responding to a chemical concentration is derived and a LC_{50} is estimated with 95% confidence limits (Cooney, 1995). Where the data did not fit the assumptions of the Probit method, the Trimmed Spearman-Kärber (TSK) method was used to calculate LC_{50} values. The use of this non-parametric method was first recommended by Hamilton *et al*. (1977) and uses interpolation to calculate the LC_{50} and the lower and upper confidence intervals. A 20% trim of data was decided upon as the upper acceptable limit of percentage trim[1]. Where the Probit method was used, the calculated and tabulated Chi squared values were reported. Where the TSK method was used, the percentage trims used were reported.

2.2.6 Species sensitivity distribution

Species sensitivity distributions were used to compare the tolerances of South African freshwater macroinvertebrates and international freshwater taxa. The SSDs were generated using the BurrliOZ computer software package (Campbell *et al*., 2000) and the Burr Type III distribution. The advantages of using the BurrliOZ software and the Burr Type III distribution to derive SSDs are discussed in chapter 3. Separate SSDs were generated for both salts using NaCl and Na_2SO_4 acute LC_{50} data extracted from the USEPA AQUIRE (USEPA, 2002) database. These data included toxicity data for vertebrates i.e. fish and were extracted using the following steps (Warne, 2001):

1. A search was conducted for response data for both aquatic flora and fauna, for sodium chloride and sodium sulphate, using the 'quick database query' function.
2. Data were copied onto an Excel spreadsheet (separately for each salt) and data for seawater organisms were eliminated.
3. Acute data were used in making comparisons. Acute data were defined as having an experiment duration between 24 and 96 hours.
4. Concentrations reported as ranges or percentages were excluded.
5. Where more than one data point existed for one species, the geometric mean of all the concentrations for that species were calculated.

Acute LC_{50} data for South African taxa of fish and invertebrates were plotted on the SSD curve for international data. Where there was more than one LC_{50} value for a

[1] A 15-20% trim is considered acceptable when using field-collected organisms (DWAF, 2000a).

species, the geometric mean of all values for a single species was used. Sources of acute toxicity data other than those generated by this study include the IWR-UCEWQ database (experiments yielding >10% mortality were excluded), Rand Afrikaans University (RAU) laboratories, a MSc thesis (O'Brien, 2004) and an honours thesis (Tyson, 1993). Data from the two theses were screened using a scoring system used by Warne (2001), adapted from the USEPA (2004) (further details provided in chapter 3). Tables 3.3 and 3.4 in chapter 3 provide comprehensive summaries of all acute LC_{50} data used.

2.3 Results

2.3.1 Water quality

Table 2.3 shows measured field electrical conductivities for both lotic and lentic collection sites. EC values average at 27.3 mS/m and 10.9 mS/m for the Kat and Balfour Rivers respectively. Dräger dam exhibited significantly higher ECs averaging 214.1 mS/m (± 59.2).

Table 2.3: Measured field electrical conductivities for all collection sites

Lotic collection sites			Lentic collection sites		
Field sample date	**Exp. No.**	**EC (mS/m)**	**Field sample date**	**Exp. No.**	**EC (mS/m)**
Kat River			**Dräger Dam**		
04/03/2003	1	30.4	03/04/2003	2	144.0
11/11/2003	9	24.2	15/04/2003	3	173.0
Balfour River			06/05/2003	4	no data
04/03/2003	1	11.8	09/09/2003	5	225.0
11/11/2003	9	10.8	23/09/2003	6	230.3
27/01/2004	10	10.1	14/10/2003	7	no data
Buffalo River			28/10/2003	8	298.0
28/02/2004	11	8.3			

(Exp. No. = Experiment Number)

Select water quality variables in each experimental vessel was monitored daily. For each experimental vessel within each experiment, the 96 hour mean EC (in mS/m), pH, temperature (Temp in ºC) and DO (in mg/L) values are summarised in appendix 2.

Water quality data provided by Resource Quality Services-DWAF for major inorganic constituents and trace metals are reported as ranges in appendix 3. Data for experiments 2-4 were not available as these samples were lost in transit.

2.3.2 Data screening

Nominal concentrations plotted against mean measured EC values using regression analysis yielded p-values >0.05 for experiment 4 with NaCl (table 2.4). A scatterplot of measured EC values vs. nominal concentrations for both salts showed that measured ECs for experiment 4 with NaCl were inconsistent with nominal concentrations for more than one channel (figures 2.11 and 2.12), and hence all results from this experiment were rejected.

Table 2.4: Results of regression analysis for NaCl and Na_2SO_4

NaCl				Na_2SO_4			
Exp No.	**r^2**	**df**	**p**	**Exp No.**	**r^2**	**df**	**p**
1	0.9802	1.57	0.0139	1	0.9970	1.53	0.0000
2	0.9939	1.38	0.0000	3	0.9448	1.54	0.0000
3	0.9448	1.42	0.0000	4	0.4246	1.38	0.0000
4	0.6317	1.38	0.3199	5	0.9904	1.34	0.0000
5	0.9993	1.35	0.0000	6	0.9868	1.38	0.0000
6	0.9970	1.37	0.0000	7	0.9873	1.53	0.0000
7	0.9960	1.53	0.0000	8	0.9925	1.34	0.0000
8	0.9926	1.34	0.0001	9	0.9951	1.42	0.0000
9	0.9968	1.38	0.0000	10	0.9927	1.43	0.0000
11	0.9959	1.38	0.0000	11	0.9970	1.38	0.0000

(Exp. No. = Experiment Number)

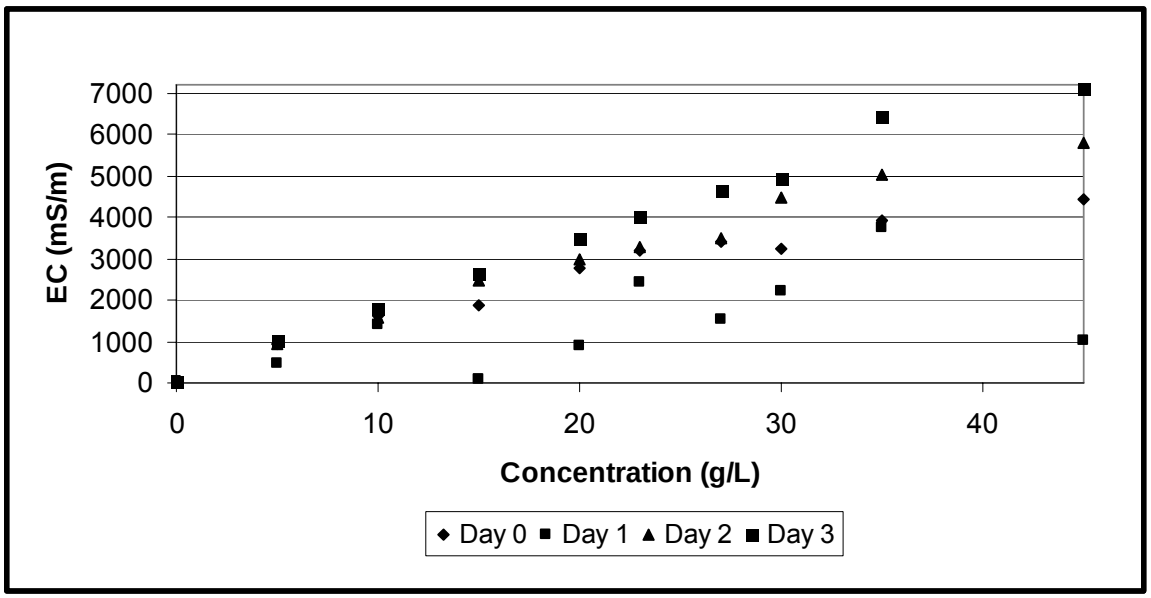


Figure 2.11: Daily measured EC readings vs. nominal concentrations for experiment 4 with NaCl

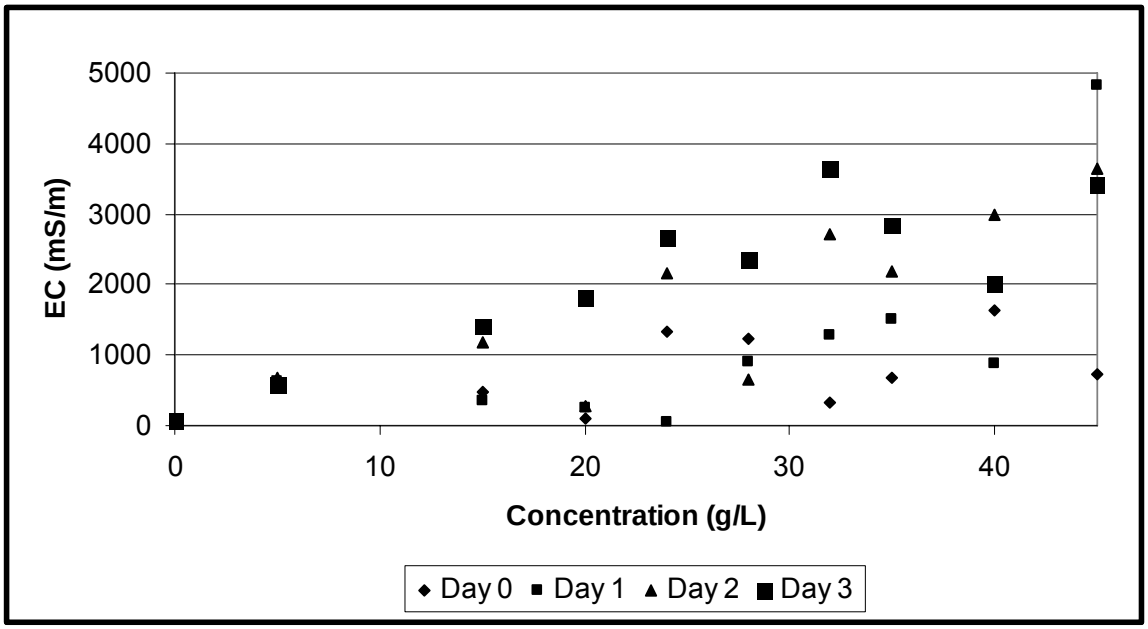


Figure 2.12: Daily measured EC readings vs. nominal concentrations for experiment 4 with Na_2SO_4

2.3.3 Organism response data

A total of 18 new datasets were generated for NaCl including data for 10 new species not previously used in toxicity tests (table 2.5) and one species previously used in toxicity tests but which was tested in this study with NaCl for the first time (*B. stenochorias*). Only one species yielded no data, that being Simulidae in experiment 11. After data screening and exclusion of datasets exhibiting unsuitable control mortalities (table 2.7), 14 datasets remained for 11 species exposed to NaCl.

A total of 18 new datasets were generated for Na_2SO_4 including data for 7 new species not previously used in toxicity tests (table 2.6) and three species previously used in toxicity tests but which were tested in this study with Na_2SO_4 for the first time (*Afronurus barnardi, Burnupia stenochorias* and *Euthraulus elegans*). After data screening and exclusion of datasets exhibiting unsuitable control mortalities (table 2.7), 15 datasets remained for 9 species, excluding the species *Demoreptus natalensis*.

Experiments that exhibited >10% mortality in the controls during tests are summarized in table 2.7. A complete list of control mortalities is provided in appendix 4.

Table 2.5: ***Summary of experimental types used in 96 hour toxicity tests conducted with NaCl showing species used for each experiment and collection sites***

Experiment No.	Experiment type	Site	Species
1	Channels	Kat	*Euthraulus elegans*
		Balfour	**Oligoneuropsis lawrencei*
		Balfour	**Demoreptus natalensis*
		Balfour	**Baetis harrisoni*
2	Glass tanks	Dräger Dam	*Coenagrionidae (RF)
3	Glass tanks	Dräger Dam	**Cloeon virgilae*
		Dräger Dam	**Plea pullula* (RF)
4	Plastic tubs	Dräger Dam	*Coenagrionidae
5	Glass tanks	Dräger Dam	**Leptoceris* sp. (RF)
		Dräger Dam	**Plea pullula*
6	Plastic tubs	Dräger Dam	**Leptoceris* sp.
7	Glass tanks	Dräger Dam	**Plea pullula*
8	Plastic tubs	Dräger Dam	*Coenagrionidae
9	Channels	Balfour	**Baetis harrisoni*
		Balfour	**Demoreptus natalensis*
		Kat	***Burnupia stenochorias*
11	Glass tanks	Buffalo	*Athericidae
	Channels	Buffalo	*Simulidae
		Buffalo	**Caenid* sp.1

*Species/organisms not previously used in toxicity tests
**Species has been used previously in toxicity tests, but is tested in this study with NaCl for the first time

Table 2.6: Summary of experimental types used in 96 hour toxicity tests conducted with Na_2SO_4 showing species used for each experiment and collection sites

Experiment No.	Experiment type	Site	Species
1	Channels	Kat	****Euthraulus elegans*
		Kat	****Afronurus barnardi*
		Kat	*Tricorythus discolor*
		Kat	****Burnupia stenochorias*
3	Glass tanks	Dräger Dam	**Cloeon virgilae*
		Dräger Dam	**Plea pullula* (RF)
		Dräger Dam	*Coenagrionidae (RF)
4	Plastic tubs	Dräger Dam	*Coenagrionidae
5	Glass tanks	Dräger Dam	**Leptocerid* sp. (RF)
		Dräger Dam	**Plea pullula*
6	Plastic tubs	Dräger Dam	**Leptoceris* sp.
7	Glass tanks	Dräger Dam	**Plea pullula*
8	Plastic tubs	Dräger Dam	*Coenagrionidae
9	Channels	Balfour	**Baetis harrisoni*
		Kat	****Euthraulus elegans*
		Kat	****Burnupia stenochorias*
10	Channels	Balfour	**Oligoneuropsis lawrencei*
		Balfour	**Demoreptus natalensis*

*Species/organisms not previously used in toxicity tests
**Species has been used previously in toxicity tests, but is tested in this study with Na_2SO_4 for the first time
RF = Range finding

Table 2.7: Experiments yielding >10% control mortalities and were subsequently excluded from the study

Experiment	Salt	Organism	Numbers responding	Numbers exposed	% control mortality
1	**NaCl**	*Baetis harrisoni*	12	25	48.0
		Demoreptus natalensis	6	8	75.0
7	**NaCl**	*Plea pullula*	22	39	56.4
	Na_2SO_4	*Plea pullula*	17	44	38.6
9	**NaCl**	*Demoreptus natalensis*	2	17	11.8*
10	**Na_2SO_4**	*Demoreptus natalensis*	9	15	60.0
11	**NaCl**	Simulidae	12	15	80.0

*This data set was not excluded as control mortality between 10-20% was still considered acceptable

The mayfly *Demoreptus natalensis* exhibited >10% control mortality for every experiment the organism was used in (experiments 1, 9 and 10), whereas *Baetis harrisoni* exhibited >10% control only in one experiment (experiment 1). Simulid larvae

exhibited both high acclimation and control mortalities, 39.29% (± 13.02%) and 80.00% respectively.

LC_{50} values for all organisms for NaCl and Na_2SO_4 are reported in tables 2.8 and 2.9 respectively. LC_{50}s for NaCl ranged from the most sensitive organisms 1807 mg/L for *Baetis harrisoni* to 24410 mg/L for Coenagrionidae and from 704 mg/L for *Oligoneuropsis lawrencei* to 29927mg/L for Coenagrionidae for Na_2SO_4. LC_{50}s could not be calculated for experiment 3 for *Plea pullula*. This was a range finding experiment and concentration ranges selected were unsuitable for the organisms to exhibit a typical sigmoidal concentration-response curve (Rand *et al*., 1995). A definitive test was completed using *Plea pullula* in experiment 5. All Probit and Trimmed-Spearman Kärber results are reported in appendices 5 and 6 respectively.

For NaCl, the 3 most sensitive taxa were all collected from the Kat River catchment, i.e. a lotic environment (*B. harrisoni, B. stenochorias* and *D. natalensis*). Of the 4 least sensitive taxa, 3 of these were collected from Dräger Dam, a lentic environment (*P. pullula,* Leptoceridae and Coenagrionidae), and all had LC_{50}s significantly different from the 3 most sensitive taxa collected from the Kat River. For Na_2SO_4, of the 6 most sensitive taxa, 5 of these were collected from the Kat River catchment (*O. lawrencei, B. harrisoni, B. stenochorias, A. barnardi* and *E. elegans*). Of the 4 least sensitive taxa for this salt, 3 were collected from Dräger Dam (*P. pullula,* Leptoceridae and Coenagrionidae), of which two (Leptoceridae and Coenagronidae) had LC_{50}s significantly different from 3 of the most sensitive taxa (*O. lawrencei, B. harrisoni* and *B. stenochorias*).

Table 2.8: Summary of LC_{50} data for NaCl

Organism	Exp. No.	LC50	Upper and Lower Confidence Limits (UCL/LCL)		Method of LC50 derivation	^{z}calculated	^{z}tabulated	% Trim
Baetis harrisoni	9	1695.00	1062.00	LCL				
			2205.00	UCL	Probit	10.03	12.59	16.67
Burnupia stenochorias	9	3899.00	3572.00	LCL				
			4299.00	UCL	Probit	9.28	12.59	
Demoreptus natalensis	9	3800.00	3320.00	LCL				
			4340.00	UCL	TSK			0.83
Cloeon virgilae	3	4682.19	4282.01	LCL				
			5119.78	UCL	TSK			1.96
Caenid sp.1	11	4800.00	3130.00	LCL				
			7350.00	UCL	TSK			6.25
Oligoneuropsis lawrencei	1	4815.67	4286.10	LCL				
			5314.81	UCL	Probit	1.67	14.10	
Leptoceridae	5	5620.00	4430.00	LCL				
			7140.00	UCL	TSK			0.00
Euthraulus elegans	1	6674.82	5177.52	LCL				
			8605.13	UCL	TSK			6.45
Plea pullula	5	6740.00	5220.00	LCL				
			8710.00	UCL	TSK			0.00
Leptoceridae	6	8290.00	7530.00	LCL				
			9120.00	UCL	TSK			0.00
Plea pullula	3			LCL				
				UCL	*			
Suragina sp.	11	18606.00	14074.00	LCL				
			25952.00	UCL	Probit	4.88	11.07	
Coenagrionidae	8	20056.00	18945.00	LCL				
			21046.00	UCL	Probit	1.43	12.59	
Coenagrionidae	4	21397.26	20300.01	LCL				
			22553.81	UCL	TSK			0.00
Coenagrionidae	2	24410.00	21880.00	LCL				
			27220.00	UCL	TSK			0.00

***Mimimum required trim was too large (81.2%), therefore LC_{50} not be calculated**

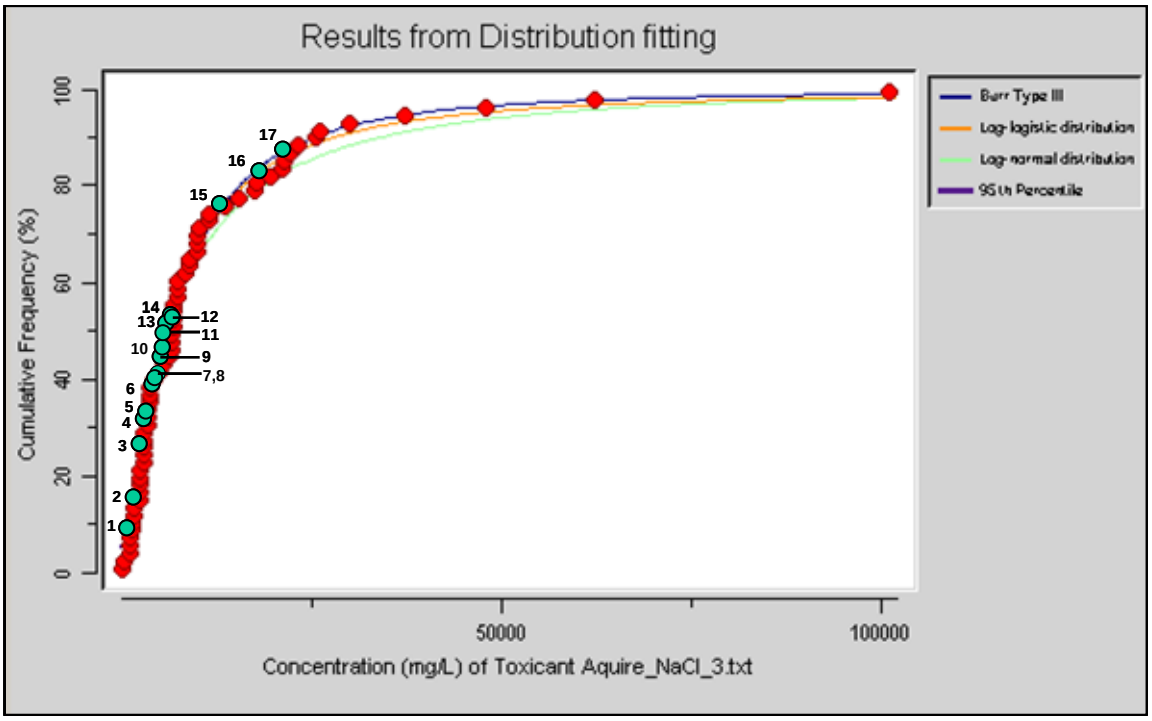
Table 2.9: Summary of LC_{50} data for Na_2SO_4

Organism	Exp. No.	LC50	Upper and Lower Confidence Limits (UCL/LCL)		Method of LC50 derivation	$\cong^2$ calculated	$\cong^2$ tabulated	% Trim
Oligoneuropsis lawrencei	10	704.31	482.74	LCL				
			1075.26	UCL	Probit	1.68	11.07	
Cloeon virgilae	3	3368.59		LCL				
				UCL	TSK			1.52
Baetis harrisoni	9	3647.38	3091.39	LCL				
			4303.37	UCL	TSK			15.79
Burnupia stenochorias	1	4580.17	3786.45	LCL				
			5540.28	UCL	TSK			0.00
Burnupia stenochorias	9	5272.00	4836.00	LCL				
			5801.00	UCL	Probit	7.13	11.07	
Afronurus barnardi	1	5979.14	4873.85	LCL				
			7192.65	UCL	Probit	4.02	14.07	
Euthraulus elegans	1	7907.97	7271.97	LCL				
			8599.60	UCL	TSK			17.86
Euthraulus elegans	9	9264.77	8759.17	LCL				
			9799.56	UCL	TSK			6.85
Plea pullula	3			LCL				
				UCL	*			
Plea pullula	5	9355.00	7117.00	LCL				
			11487.00	UCL	Probit	3.76	11.07	
Tricorythus discolor	1	9402.13	8234.00	LCL				
			12187.46	UCL	Probit	7.13	14.07	
Leptoceridae	5	9720.00	7910.00	LCL				
			11950.00	UCL	TSK			4.00
Leptoceridae	6	11345.00	10555.00	LCL				
			12343.00	UCL	Probit	5.82	14.07	
Coenagrionidae	8	23609.00	22633.00	LCL				
			24505.00	UCL	Probit	3.57	12.59	
Coenagrionidae	4	26224.00	25026.00	LCL				
			27365.00	UCL	Probit	6.89	14.07	
Coenagrionidae	3	29 926.71	24 223.67	LCL				
			36 972.42	UCL	TSK			14.29

***Mimimum required trim was too large (90.3%), therefore LC50 not be calculated**

2.3.4 Species sensitivity distribution

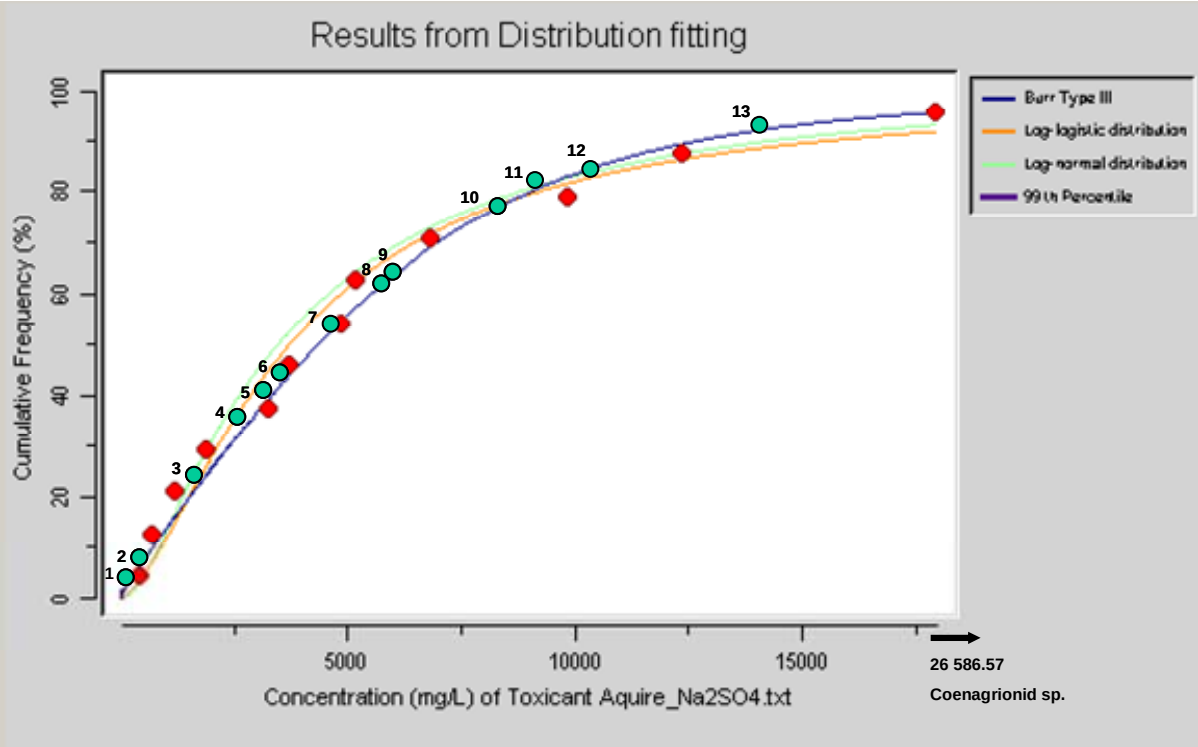
Summaries of the data extracted from the USEPA AQUIRE database are given in appendix 7. Figures 2.13 and 2.14 show the SSDs generated by the BurrliOz software for NaCl and Na_2SO_4 respectively, using data from the USEPA AQUIRE database. The figures also indicate where along the distribution the tolerances generated from the South African macroinvertebrate taxa lie. Where there was more than 1 datum for a single species, the geometric mean was calculated and incorporated in the SSD.



1 – ***Oreochromus mossambicus*** **(V)**
2 – ***Baetis harrisoni***
3 – ***Daphnia pulex***
4 – ***Demoreptus natalensis***
5 – ***Burnupia stenochorias***
6 – ***Cloeon virgilae***
7 – ***Caenid*** **sp.1**
– ***Oligoneuropsis lawrencei***
8 – ***Afronurus barnardi***
– ***Tricorythus discolor***
9 – ***Clarias gariepinus*** **(V)***
– ***Barbus trimaculatas*** **(V)***
10 – ***Afronurus peringueyi***
11 – ***Caridina nilotica***
– ***Adenophlebia auriculata***
12 – ***Plea pullula***
- ***Leptocerid sp.***
- ***Euthraulus elegans***
13 – ***Afroptilum sudafricanum***
14 – ***Tilapia sparmani*** **(V)***
15 – ***Paramelita nigroculus***
16 – ***Suragina*** **sp.**
17 - Coenagrinidae

***V – denotes vertebrate**

Figure 2.13: ***SSD for NaCl generated using acute data from the USEPA AQUIRE database. Acute LC_{50}s for South African species are indicated and listed (1-17) showing the spread of sensitivities relative to the international database***



1 – ***Clarias gariepinus*** **(V)***
2 – ***Oligoneuropsis lawrencei***
3 – ***Daphnia pulex***
4 – ***Tricorythus discolor***
5 – ***Cloeon virgilae***
6 – ***Baetis harrisoni***
7 – ***Burnupia stenochorias***
8 – ***Afronurus barnardi***
9 – ***Caridina nilotica***
10 – ***Euthraulus elegans***
11 – ***Plea pullula***
12 – ***Leptocerid*** **sp.**
13 – ***Tilapia sparmani*** **(V)***

***V – denotes vertebrate**

Figure 2.14: ***SSD for Na_2SO_4 generated using acute data from the USEPA AQUIRE database. Acute LC_{50}s for South African species are indicated and listed (1-17) showing the spread of sensitivities relative to the international database***

2.4 Discussion

2.4.1 Water quality

Due to the fact that varying water quality parameters can introduce extraneous variability in the test endpoints (Cooney, 1995), selected water quality parameters were measured for the duration of each experiment (appendix 2 and 3). Mean standard deviations for each experiment were low for pH, temperature and DO readings varying from 0.1 to 0.2, 0.1 to 0.8 and from 0.3 to 1.9 respectively. This shows that variability caused by extraneous factors was kept to a minimum.

The major inorganic determinant and trace metal data provided by the Resource Quality Services (RQS) were reported as ranges in appendix 3. These upper limits for each constituent were compared to the target water quality ranges (TWQRs) provided by the South African Water Quality Guidelines in volume 7 for aquatic guidelines (DWAF, 1996a). In some instances the TWQRs were exceeded in certain channels/tubs/tanks. Concentration-response data from these experimental vessels were only excluded where the data did not meet the assumptions of the statistical models. As a result no data were excluded based on the results of RQS water quality analyses.

2.4.2 Aquatic invertebrates in aquatic toxicology

A range of test organisms can be used for toxicity tests. These can include both field-collected and laboratory-reared organisms. Rand *et al.* (1995) outlines six criteria for the selection of test organisms:

1. Because sensitivities vary among species, a group of species representing a broad range of sensitivities should be used whenever possible.
2. Widely available and abundant species should be considered.
3. Whenever possible, species should be studied that are indigenous to or representative of the ecosystem that may receive the impact.
4. Species that are recreationally, commercially or ecologically important should be included.
5. Organisms should be amenable to routine maintenance in the laboratory and techniques should be available for culturing and rearing them in the laboratory so as to facilitate both acute and chronic toxicity tests.
6. If there is adequate background information on a species, the data from a test may be more easily interpreted.

Laboratory-reared organisms routinely used in toxicity tests are known as ‘standard laboratory organisms’ (Cooney, 1995). Typical examples include water fleas (*Daphnia*) and guppies (*P. reticulata*) and are used for routine toxicity testing (DWAF, 2000a). Due to the common use of laboratory-bred organisms and the existence of protocols using standard laboratory organisms, international toxicological data are dominated by response data generated by these organisms.

Aquatic invertebrates have been identified as useful indicators of water quality (DWAF, 2000a; Patrick and Palavage, 1994). Wright & Welbourn (2002) identified particular

species of aquatic invertebrates as indicator species. Hence, these species could be utilised as indicators of environmental conditions at the 'whole organism' level (for particular ecosystems). These species included: mayfly larvae (*Baetis rhodani and Hexagenia limbata*), Diatoms, tubificid oligochaetes (*Minodrilus* spp.), walleye (*Stizostedion vitreum*), amphipod (*Pontoporeia hoyi*) (Wright & Welbourn, 2002). Cooney (1995) outlines a suite of test organisms that should be used in freshwater tests, wherever possible. Of the invertebrates suggested, species included Daphnids, amphipods, crayfish, stoneflies, mayflies, midges, snails, planaria and rotifers (Cooney, 1995).

Laboratory-reared organisms are preferably used in toxicity tests as they are made easily available through stock cultures, their biology and physiology is often well-understood and they don't introduce as much genetic variability as do field-collected organisms. However, standard test protocols using laboratory-reared organisms indigenous to South Africa are limited and protocols for culturing indigenous test organism in South Africa are in the process of being developed and refined. Palmer (2002) reports no existing South African facility for the breeding of indigenous invertebrates for routine toxicity tests. This has prompted the use of wild-caught indigenous organisms, such as riffle-dwelling macroinvertebrates in toxicity tests, as has been used in this study. Such tests can be used to generate site-specific toxicity data as well as data that can be used to formulate WQGs that are directly applicable to South African aquatic ecosystems (Palmer, 2002).

Macroinvertebrates indigenous to South Africa have been used in toxicological experiments. For example, Palmer and Scherman (1998) used selected macroinvertebrates from the Sabie River in the Kruger National Park. Tyson (1993) conducted acute and chronic tests with sodium chloride on a mountain stream amphipod, *Paramelita nigroculus*, collected from the Skeleton Gorge Stream in the South-Western Cape. Musibono (1998) observed the combined toxic effects of aluminium, copper and manganese on the same species. The Unilever Centre for Environmental Water Quality and the Institute for Water Research (UCEWQ-IWR) at Rhodes University, Grahamstown, South Africa, has conducted various toxicological experiments with a range of macroinvertebrate species, including many salinity-based experiments (Palmer et. al., in press b). As a result of this work, a protocol was developed for 'Acute Toxicity Testing using Selected Riverine Invertebrates in Artificial Stream Systems' (DWAF, 2000a). This protocol served as the main experimental method for this study and was adapted where necessary.

2.4.3 The suitability of South African indigenous macroinvertebrates used in this study for acute toxicity tests

Experiments conducted with certain organisms did not provide a typical dose-response relationship, and hence did not lend themselves towards selected standard regression analytical techniques. These included experiments conducted with the baetid *Demoreptus natalensis*, the bug *Plea pullula* and the simulid larvae. According to DWAFs protocol for using riverine invertebrates in acute toxicity tests, baetids should only be used if other test organisms are not available, as they often occur in species complexes (DWAF, 2000a). The high control mortalities observed for the baetids can be attributed to handling stress and the lack of suitable numbers of organisms per experimental vessel (at least 40 organisms per experimental vessel is recommended where organisms represent a species complex; DWAF, 2000a). Although *Baetis harrisoni* exhibited >10% mortality for one experiment in this study, *Baetis harrisoni* collected from lotic aquatic systems, have successfully been used in previous toxicity tests (Binder, 1999, Williams, 1996; Williams *et al.*, 2003). Because baetids require extra care and minimal handling, it is suggested that collecting trips are allocated to the collection of baetids only at any one time (instead of various different organisms being collected) per sampling trip so as to minimise collecting time and handling stress.

LC_{50} values could be calculated for *Plea pullula* (excluding the range finding experiment no.3), however, the concentration response curves continued to display inconsistencies in following a typical sigmoidal curve. This is possibly due to the fact that they are air-breathing organisms (Gerber and Gabriel, 2002) and were exhibiting some form of behavioural avoidance towards the toxicant. Some pleids also became positively buoyant as a result of handling, inhibiting swimming. If pleids are to be considered for future acute toxicity tests, handling and test methods would need to be refined so as to minimize variability in results.

If simulids are to be considered in future tests, the method of collection and the nature of the experimental vessel would need to be reviewed. For example, the method of larval transportation as described by De Moor (2002) is for rearing purposes and might not be suitable for toxicity purposes. Furthermore, simulidae are found in very fast flowing reaches (Davies and Day, 1998c) of a river and the recirculating channels might not provide the most suitable experimental habitat for the larvae. Experiment 3 did not yield a LC_{50} for *Plea pullula* as this experiment was used as a range finding

experiment and the concentration range selected was not suitable to generate a typically sigmoidal concentration response curve.

Most of the test organisms in this study proved to be suitable test organisms with the exception of Pleiidae and the simulid larvae, and in some instances baetid larvae. Repeated toxicity testing on individual species would help assess the variability in responses introduced from field-collected organisms. Variability is inherent in biological organisms, particularly if indigenous field-collected organisms are used for toxicity testing (Scherman *et al*., 2001). For this reason, a replicated regression design is a recommended experimental design (DWAF, 2000a). However, this option was not available in this study due to limited organism numbers in the field. Some attempts have been made to quantify the degree of variability introduced when using field-collected organisms (Scherman *et al.*, 2001). Regardless, many questions posed around variability of organisms responses remain unanswered due to limited availability of data. Instead, an approach was used in this study as recommended by Scherman *et al.* (2001) which relies on other parameters for checking data validity. These include monitoring control mortalities, observing warning messages yielded by the Probit model and checking percentage trims implemented by the Trimmed Spearman-Kärber model. This study also provides a valuable platform for further development of experimental methods using invertebrates collected from lentic aquatic environments.

2.4.4 Comparative acute salinity responses

Acute salinity responses between taxa were examined so as to identify possible relationships between taxa and acute responses to salinity. The results show that more sensitive taxa were collected from lotic aquatic environments and that more tolerant taxa were collected from Dräger Dam, a lentic environment (table 2.8 and 2.9). The fact that more salt tolerant taxa were sourced from a lentic environment may be indicative of lentic organisms having an inherent tolerance to an aquatic environment characterized by high salinities (table 2.3), especially in periods of low rainfall and high evaporation rates, when salinity levels may reach very high levels (table 2.3 shows EC increased from 144 mS/m in early April to 298 mS/m in late October).

Three main sources of literature exist that might allow for some comparison of salt tolerance data. These include Hart *et al.* (1991), Kefford *et al.* (2003) and the AQUIRE database of toxicology data (USEPA, 2002). Hart *et al.* (1991) reviewed the salt sensitivity of Australian freshwater biota including invertebrates and reported the range

of field salinities that are inhabited by various invertebrates and, where possible, their relative abundances. Kefford *et al*. (2003) conducted 72hr acute toxicity tests using Ocean Nature salt and reported all LC_{50} values as EC (in mS/cm). Ocean Nature salt has a similar ionic composition to sea water, which is sodium chloride dominated (Kefford, 2000). The AQUIRE database reports <48hr, 48hr and 96hr LC_{50}s in mg/L for both NaCl and Na_2SO_4 as well as other salts. Problems arise when trying to compare South African salinity tolerance data with international data as there is no international standard for reporting salinity. Salinity can be reported in EC (mS/m, μS/m, mS/cm) or in TDS (ppm, mg/L, g/L)[1]. Conversions from EC to TDS exist [TDS (mg/L) = EC (mS/m at 25 °C) x 6.5, DWAF, 1996a), however inaccuracy could be incurred in converting values where the ionic composition of a solution is unknown as the factor of 6.5 is influenced by the ionic make-up of dissolved solids and can range from 4.0 to 9.0 (Palmer *et al*., 2004a). Some work has been done to derive conversions for individual salts using regression equations (Palmer *et al*., 2004a). Despite these confounding factors, some comparisons are shown in table 2.10, comparing LC_{50} data for taxa which are common to both the AQUIRE database, Kefford *et al*. (2003) and this study. These taxa include baetids (Ephemeroptera), other Ephemeroptera, Trichoptera, Hemiptera and Odonata. Baetids are separated from the order Ephemeroptera as they are a diverse family comprised of many species complexes (Barber-James, pers comm.). LC_{50}s reported in mg/L from the AQUIRE database and this study were converted to mS/m using the conversion:

- EC (mS/m) = TDS (mg/L) ÷ 6.5 (DWAF, 1996a)

Between taxa, salinity responses from this study followed similar trends to the other two studies. LC_{50} values for all the taxa for each study ranged between 550 and 6000 mS/m (Kefford *et al*., 2003a), 278 and 3755 mS/m (for NaCl for this study) and between 1032-3950 mS/m (AQUIRE database). Baetids proved to be the most salt sensitive taxa with the lowest range of LC_{50}s (table 2.10), followed by other Ephemeroptera (excluding baetids), Trichoptera and Hemiptera.

[1] 1 mS/cm = 100 mS/m = 1000 μS/cm = 0.65 ppt = 650 ppm = 650 mg/L (TDS)

Table 2.10: LC_{50} values for taxa common to three salinity tolerance studies (all values reported in mS/m)

Taxa	Kefford et. al., 2003 72hr LC50 with Ocean Nature salt	This study		Other studies (from Kefford, et. al. 2003)	AQUIRE database (NaCl)
		NaCl	Na_2SO_4		
Baetidae	**550-620** ($\bar{\mu}$=585, n=2)	**278-739** ($\bar{\mu}$=580, n=4)	**43-561** ($\bar{\mu}$=374, n=3)	**100-1400**	**1032** (n=1)
Ephemeroptera	**>1260-1500** (*, n=2)	**748-1027** ($\bar{\mu}$=887, n=2)	**108-1447** ($\bar{\mu}$=1022, n=5)	**1400-2000**	**385** (n=1)
Trichoptera	**>320->2560** (*, n=13)	**865-1275** ($\bar{\mu}$=1070, n=2)	**1495-1745** ($\bar{\mu}$=1620, n=2)	**930-3400**	**869-1385** ($\bar{\mu}$=1019, n=3)
Hemiptera	**1500-4700** (*, n=7)	**1037** (n=1)	**1439** (n=1)	**1800**	
Odonata	**>12 600-6000** (*, n=7)	**3085-3755** ($\bar{\mu}$=3378 n=3)	**3632-4604** ($\bar{\mu}$=580 n=3)	**2000-2100**	**3950** (n=1)

* Means not reported as LC_{50}s were reported as ranges

Odonata exhibited the highest tolerance to salinity in all three studies, with one species of Odonata from the AQUIRE database exhibiting the highest LC_{50} of 3950 mS/m. Similarly, Hart *et al*. (1991) reports finding Odonata present in field salinities of up to 3230 mS/m, however, Hart *et al*. (1991) also reports that, with a few exceptions, Odonata are likely to be relatively salt sensitive. The exceptions include Ischnura, a genus of damselflies, and are the likely reason for the high values reflected for Odonata in this study in table 2.10. In the study conducted by Kefford *et al*. (2003), 3 of the 6 species comprising the mean LC_{50} are represented by damselflies, all 3 of which comprised 6 of the most salt tolerant species tested by Kefford *et al*. (2003) In this study, all values comprising the LC_{50} value for Odonata are represented by the Coenagrionid family of damselflies. Hence, it is possible that damselflies represent some of the most salt tolerant species of the order Odonata and current available data suggest that other Odonata species are not as salt tolerant.

The SSDs for NaCl and Na_2SO_4 (figures 2.13 and 2.14) show that species tolerances from this study fall within the range of tolerances of species in the AQUIRE database for both salts, with the exception of Coenagrionidae for Na_2SO_4 . The Sailfin molly fish, *Poecilia latipinna*, from the AQUIRE database had the highest geometric mean LC_{50} of 2755 mS/m for Na_2SO_4 and the *Coenagrionid* sp. from this study had a geometric mean LC_{50} of 4090 mS/m.

Acute salinity responses were also examined between broader taxonomic groupings. Two data sets were used to facilitate these comparisons. The first was extracted from Kefford *et al*. (2003) and acute response data for Ocean Nature salt summarised in

table 2.11. The second set of data were extracted from the USEPA AQUIRE database (table 2.12) (the UCEWQ database was not used as this database is dominated by insects). Acute data for NaCl with experiment durations of ≥48 hours and ≤96 hour were extracted. Only NaCl tolerance data were extracted as Ocean Nature salt is largely composed of NaCl, and would allow for the most obvious comparisons (Ocean Nature is a commercially available mixture of salts that simulates the ionic composition of seawater). Geometric means for endpoints were calculated, firstly where two or more species were present, and then for three broad taxonomic groups, i.e. non-arthropods, insects and mites (arthropods), and crustaceans (arthropods) (tables 2.11 and 2.12).

Table 2.11: Summary of LC_{50} (72 hour) data for Ocean Nature from Kefford et al. (2003)

Taxonomic grouping	**Mean LC_{50} (mS/m)**	**LC_{50} range (mS/m)**	**Sample size (n)**
Non-arthropod	1200	900-1400	11
Insects and mites	3000	550-5500	41
Crustaceans	5700	3800-7600	5

Table 2.12: Summary of acute toxicity data from the AQUIRE database (experiment durations between 48-96 hour) for NaCl

Taxonomic grouping	**Geometric mean LC_{50} (mS/m)**	**LC_{50} range (mS/m)**	**Sample size (n)**
Crustaceans	569	900-1400	8
Non-arthropods	1011	368-4334	20
Insects	1471	385-9571	9

Kefford *et al*. (2003) reported that the most striking differences in mean LC_{50}s were between arthropods and non-arthropods, with non-arthropods being the most sensitive, insects and mites less sensitive and crustaceans the most tolerant (table 2.11). When examining the AQUIRE data for sodium chloride, crustaceans were found to be the most sensitive, non-arthropods less sensitive and insects the most tolerant taxonomic group (table 2.12). Certain organisms might be exhibiting higher tolerances to the Ocean Nature salt due to the ionic make-up of the salt, that is, it is not pure NaCl and the ionic composition of the salt might be ameliorating toxic effects. For example, ions such as Na^+ and K^+ are important for osmo-regulatory processes in aquatic organisms (Chapman, 1998). A possible reason for crustaceans being the most tolerant to Ocean

Nature salt might be due to the fact that, given that it mimics sea salt (Kefford *et al*., 2003b) and that many crustaceans are of marine origin, many crustaceans may have inherent osmoregulatory capabilities to the Ocean Nature salt.

However, the value of comparing tolerances on a broader taxonomic level may be limited due to higher variability in responses introduced by grouping species on a broader level. Scherman *et al*. (2001) note how various factors can contribute to increasing variability in organism response data. These include the test organisms (biological variability), the toxicant, the diluent used, the area and river of origin of the test organisms and the experimental design (Scherman *et al*., 2001). This high degree of natural and biological variability further increases the difficulty associated with such comparative studies.

The process of accurately quantifying the effect of anthropogenically-induced salinisation on aquatic ecosystems is complex. In addition to the variability associated with laboratory testing, many other field-related factors introduce variability. These are associated with naturally occurring environmental and biological interactions and include factors such as ionic behaviour of salts, variables such as temperature, turbidity and flow regimes, as well as ecological factors such as prey-predator interactions (Hart *et al*., 1990).

The ionic composition of different salts is known to affect living organisms differently (Mount *et al*., 1997) and individual salts are known to vary in toxicity (Jooste and Rossouw, 2002, Palmer *et al*., 2004a). This has highlighted the need for water resource managers to establish the ionic composition of water bodies, to facilitate a more accurate approach in water quality management. The Department of Water Affairs and Forestry (DWAF) routinely monitor ionic composition at monitoring points around the country. Jooste and Rossouw (2002) have developed the Toxciologically Important Major Salts (TIMS) method in South Africa which models potential salt concentrations given ionic concentrations (chapter 4). Such a method is useful but limits the management of water quality to the location or in the near vicinity of a DWAF weir. Measuring EC remains an easier and cheaper method of monitoring salinity. New methods have been developed for South Africa which propose how EC can be used in water quality management as part of a Reserve determination in the absence of any other water quality data (Palmer *et al*., 2004a). These methods demonstrate how measured EC can be related back to the ecological Reserve categories of the resource

classification system, provided the most toxic salt, magnesium sulphate ($MgSO_4$) is not likely to be present (Palmer *et al*., 2004a).

A further complicating factor in the process of quantifying the toxic effects of salinity is the issue of environmental variability. Kefford *et al*. (2004) suggest that there may be changes in other water quality variables, including ionic proportions, and other stresses in the aquatic environment that when combined with elevated salinity results in salinity tolerances different to those indicated by LC_{50} values. Furthermore, certain species might be affected indirectly by salinisation affecting their habitat or more salt-sensitive prey, predators, competitors and parasites (Kefford *et al*., 2004).

2.5 Conclusion

The need to understand chronic effects of salinity on aquatic ecosystems should not be under-estimated as these could have much broader impacts on aquatic ecosystems as a whole. These impacts could include a decrease in biodiversity, lowering in productivity and changes in the natural characteristics of aquatic ecosystems (Williams, 1999), as well as a breakdown of food-web structure, loss of riverine habitat and overall environmental degradation (Bailey and James, 2000). The chronic and sub-lethal effects of long-term exposure of lower concentrations of salts on invertebrates and other aquatic organisms are not well-documented (Hart *et al*., 1990, Kefford, 1998). This is because chronic toxicity tests are expensive, time consuming and complex to conduct (Coonery, 1995). Instead acute response data are often used to extrapolate chronic responses. Hence, this study makes a valuable contribution towards those considering undertaking acute to chronic extrapolations.

This study has also shown that invertebrates are sensitive to two inorganic salts and can exhibit a wide range of tolerances to salinity. When comparing South African invertebrate taxa according to order, they follow similar trends in salinity responses compared to international taxa.

It is recommended that the tolerance data generated from this study can best be applied in water resource management by using Burr Type III distributions, which include the Aldenberg and Slob distribution (Aldenberg and Slob, 1993), to derive species sensitivity distributions (SSDs), which in turn can be used to derive water quality criteria (Warne, 1998). The validity and application of this is addressed in chapter 3. Furthermore, there is a need for an international standard in reporting

salinity tolerance data so as to facilitate comparisons between similar studies. The process of developing accurate WQGs in South Africa for salinity is a complex one, but should remain under review as more data are gathered using indigenous organisms for toxicity tests and as methods for applying these data in water resource management are refined.

CHAPTER 3: The role of acute toxicity data in the derivation of water quality guidelines

3.1 Introduction

This chapter aims to use data generated in chapter 2 as well as already existing acute toxicity data to explore options for deriving WQGs for NaCl and Na_2SO_4. The role that different types of acute toxicity data play in this derivation process is examined and form the basis for recommendations made for future toxicity testing.

Internationally, WQGs serve as essential tools to assist water resource managers in managing aquatic ecosystems effectively by providing quantitative goals to aid management decisions. For example, the United States (USEPA, 1994), Canada (CCREM, 1991), the Netherlands (Van de Plassche *et al*., 1993), Denmark (Petersen and Pedersen, 1995), Australia (ANZECC, 2000) and South Africa (DWAF, 1996a) have all published WQGs. Often the data used in the guideline derivation process are dominated by acute single-species tolerance data, lacking in long-term effect, multiple-species and whole-ecosystem data. As a result, the development of methods for deriving guidelines has been steered by the nature of existing data.

The derivation of WQGs for any toxicant is a complex process and the methods used vary and have evolved over time. One of the earliest methods used in guideline development is the safety factor or assessment factor (AF) method (Warne, 1998). This method divides a reported toxicity value (usually the lowest toxicity value such as a no-observed-effect concentration) by an assessment factor which has a typical value of either 10, 100 or 1000 (Okkerman *et al*., 1991). The AF is governed by the perceived quality of the data (Warne, 1998) and the representation of the data of whole ecosystems. Sometimes the acute to chronic ratio (ACR) is used as the AF (the ratio of acute toxicity data to chronic toxicity data for a particular toxicant) which is calculated by dividing acute toxicity endpoints by chronic endpoints. Typically they must be calculated for the same species and, as recommended by Warne (2001), have been presented in the same scientific paper or at least determined in the same laboratory.

In an effort to move towards more rigorous, statistically-based methods, various authors have explored extrapolation procedures which attempt to predict boundary/trigger values (BVs or TVs) or concentrations for a certain toxicant, below

which adverse effects in ecosystems are unlikely to occur (Okkerman *et al*., 1991). These species sensitivity distribution (SSD) extrapolation methods theoretically aim to protect 95% of species (or any other stipulated percentage) represented in an ecosystem (Stephan *et al*., 1985; Wagner and Løkke, 1991; Aldenberg and Slob 1993). Extrapolation techniques are based on the concept of a distribution of tolerances of a range of species (Smith and Cairns, 1993) and are especially useful where limited data exist for few species (Pennington, 2003). This method is currently preferred to the AF method for deriving protective concentrations (Knoben *et al*., 1998, Kooijman, 1987, Newman *et al*., 2000, Okkerman *et al*., 1991, Posthuma *et al*., 2001, Schudoma, 1994) and is increasingly used in ecological risk assessment procedures (e.g. Solomon *et al*., 1996, Steen *et al*., 1999, Van Straalen, 2002).

The aim of a SSD analysis is to predict a chemical concentration protective of a pre-determined percentage of species in the environment and can therefore be used to derive WQGs. A SSD constructs a cumulative plot of logarithmically transformed toxicity endpoint data (ideally no observed effect concentration or NOEC data) against rank assigned percentiles for each toxic endpoint value to which a statistical distribution is fitted (Wheeler *et al*., 2002). Various statistical distributions can be used. A log-normal distribution proposed by Wagner and Løkke (1991) has been adopted in Europe and the United States and others include a log-logistic distribution (Aldenberg and Slob, 1993, Kooijman, 1987) and the log-triangular distribution proposed by Stephen *et al*. (1985).

This study adopts the three parameter Burr Type (BT) III distribution used in Australia and New Zealand (Wheeler *et al*., 2002) and required by the Australian Environment Protection Agency (Campbell *et al*., 2000). There are several advantages for using the BT III distribution:

1. The Burr Type III distribution has been shown to provide more accurate results than the Aldenberg and Slob log-logistic approach which tends to underestimate true protection values, especially for large sample sizes (Shao, 2000).
2. It is a very flexible distribution which can provide good approximations to many commonly used distributions, including log-logistic distribution.
3. It uses the bootstrapping resampling technique which makes it useful for small sample sizes (Shao, 2000) which is often typical of toxicological datasets.

Furthermore, there is no theoretical basis for preferring one statistical distribution over another (Smith & Cairns, 1993; Forbes & Forbes, 1993; Shao, 2000; Warne 2001).

In this study the BurrliOZ computer software developed by Campbell *et al*. (2000) was used to estimate various levels of protective concentrations (PCs) by fitting the Burr Type III distribution to various datasets. The advantage of using this software is that the required percentage of species protection can be stipulated as well as the required confidence limits. This makes it a useful tool for risk assessment studies. Typically PC95 50% values are calculated which equates to a protective concentration that theoretically protects 95% of species with 50% confidence. The nature of the input data used to derive the SSD will determine if the derived PC is theoretically protective of either all species known, or all species present in an ecosystem.

In the derivation of the Australian and New Zealand Water Quality Guidelines for toxicants, the Australian and New Zealand Environment and Conservation Council (ANZECC) used BurrliOZ to derive toxicant trigger values (TVs) at different levels of reliability, depending on availability and quality of data (ANZECC, 1992). The Australian and New Zealand Water Quality guidelines were derived using a newly developed framework and some key elements of the framework include (Warne, 2001):

- Three grades of TVs are derived: high, medium and low reliability TVs.
- The grades reflect the confidence that they will provide adequate environmental protection, depending on the quantity, type and representative-ness of available toxicity data from which they are derived. For example, the more taxonomic groups represented by a dataset, the higher the reliability of the TV.
- High reliability TVs are derived in preference to medium and low reliability TVs.
- Acute, chronic, laboratory, field, mesocosm and microcosm data are used for deriving TVs.
- Various methods can be used to derive TVs. The choice of method is decided upon using a hierarchical approach, depending on the nature of available toxicity data. The preferred statistical distribution method is the Burr Type III method developed by Shao (2000). Alternatively, a modification of the Canadian assessment factor (AF) method (CCME, 1991) is used for medium and low reliability trigger values.

The framework used in the Australian and New Zealand (A&NZ) method to derive WQGs has recently been adopted in developing pilot WQGs in South Africa for

selected organic toxicants (Warne *et al*., draft report). The A&NZ method was favoured because it is a revised method based on previous established Australian WQGs (ANZECC, 1992b), and hence adopts current world-wide trends and advances in the scientific understanding of guideline derivation. These advances include the preferred use of SSDs to the AF method, which are also evident in Denmark (Peterson and Pedersen, 1995) and the Netherlands (Van de Plaasche *et al*., 1993) and has been a preferred method of use by the USEPA (1996) and the OECD (1992).

In South Africa, the approach of refining WQGs for salinity in South African aquatic ecosystems has moved from one based on EC/TDS based guidelines (DWAF, 1996a) to setting guidelines for individual inorganic salts, accommodating for differing toxicity among individual salts. This approach is more complex, and according to Palmer *et al*. (2004a), poses significant problems for water resource managers. Firstly, salinity data for many sites are only reported in EC. Secondly, EC is readily measured and data are immediately available, whereas data for analysed ions from which probable salt concentrations can be calculated, are often delayed in reaching a manager's desk. This led to the development of "screening" WQGs for EC (based on the Australian and New Zealand framework) for use in instances where EC data are available and values low enough for certain ionic mixtures to be less critical (for example, where $MgSO_4$, the most toxic salt, is unlikely to be present) (Palmer *et al*., 2004a). The BVs for EC proposed by Palmer *et al*. (2004c) integrated various methods of guideline development, including the recent framework used to derive WQGs for Australia and New Zealand (Warne, 2001), using all data available internationally as well as South African toxicity response data.

Jooste and Rossouw (2002) developed a method for deriving benchmarks (equivalent to BVs or TVs[1]) for various water quality constituents, including 6 inorganic salts, in an effort to derive WQGs that would ensure ecological integrity in fresh surface water resources. The benchmark values derived by Jooste and Rossouw (2002) were compiled using data from the USEPA AQUIRE database. Toxicity data were extracted subject to (Jooste and Rossouw, 2002):

[1] Jooste and Rossouw (2002) derive BVs also referred to as Ecospecs or benchmarks. BVs are the numerical values for a specific toxicant which indicate whether a measured toxicant in an aquatic environment categorises the aquatic ecosystem into either a natural, good, fair or poor condition. The Australian and New Zealand framework refers to BVs as TVs. This study used the term BVs (which are derived using different PCs).

- Freshwater as medium
- Laboratory results
- Single chemical species
- LC_{50}, EC_{50}, LOEC, NOEC
- All endpoints (mortality, immobilisation, behaviour, physiological, population, etc.)

Data were refined using the following steps (Jooste and Rossouw, 2002):

- Data were rejected if experiments were flagged due to insufficient control data (blank and/or positive control).
- Endpoint values reported as “larger than” were replaced by the value.
- Concentrations were standardised to mg/L units.
- The endpoint concentrations from otherwise identical records were aggregated using the geometric mean.
- Results were aggregated to genus mean values also using the geometric mean.
- Care was taken to ensure representation of toxicity data for: a) fish, b) other aquatic vertebrates, c) invertebrates, d) plants (no distinction was made between vascular plants and algae).

Data were extrapolated to a 336 hr (2 week) LC_{50} and benchmarks derived for inorganic salts from an interpolation of the hazard-based stressor response curve generated from two points: the lethality benchmark (used to predict the natural/good boundary, figure 1.1) and the sub-lethality benchmark (used to predict the fair/poor boundary, figure 1.1) (Jooste and Rossouw, 2002). One of the main differences between the Australian and Jooste and Rossouw’s approach is in the definition of acute and chronic data. Warne (2001) defines chronic data as having an experimental duration greater than 96 hours for multi-celled organisms, and greater than 72 hours for single-celled organisms. Acute data were defined as having an experimental duration greater than 24 hours and less than the duration for chronic data (Warne, 2001), i.e. data were defined strictly in terms of exposure time. Jooste and Rossouw (2002) defined data in terms of endpoints, where a lethal endpoint equates to an acute datum and a sub-lethal endpoint equated to a chronic datum. This combined with the extrapolation to a 336 hour exposure, results in the derivation of more conservative benchmark values by Jooste and Rossouw (2002).

This chapter will explore different data manipulation methods for guideline derivation which could be used to derive inorganic salt-specific WQGs in South Africa. Data generated in chapter 2 are combined together with other sources of toxicity response data for NaCl and Na_2SO_4 available from international databases (including the IWR-UCEWQ database) and used to derive different SSDs, and hence a range of PC values. These PC values are then discussed in terms of South Africa's BVs for its ecological Reserve categories. Because toxicity response data are variable depending on experimental duration, test organism, test type, test endpoint used, etc., the application that different types of acute toxicity data play in deriving WQGs for inorganic salts is discussed, using NaCl and Na_2SO_4 as reference toxicants.

Comparisons are drawn between BVs derived using the different SSDs, the salt benchmark values proposed by Jooste and Rossouw (2002) and the EC BVs proposed in Palmer *et al*. (2004c).

3.2 Methods

The following types of NaCl and Na_2SO_4 data, from various sources, were used to derive the necessary SSDs:

1. The USEPAs international AQUIRE database (USEPA, 2002) which yielded both acute and chronic data. Acute data were defined as having an experimental duration between 24 and 96 hours inclusive (Warne, 2001). Chronic data included NOEC and LOEC values for experiments longer than 96 hours. Where more than one datum existed for the same species the geometric mean of all the values for that species was used in the SSD (Warne, 2001).
2. Acute data generated in chapter 2. All these data were 96 hour acute data and were included into the IWR-UCEWQ database.
3. Acute (lethal), sub-chronic[1] (lethal) and chronic (sub-lethal) data from the IWR-UCEWQ database, Rhodes University, Grahamstown. Acute data were defined as having an experimental duration between 24 and 96 hours (Warne, 2001). Sub-chronic data included all 10 day experiments. Chronic data consisted of experiments with an experimental duration longer than 10 days and were provided as part of a current M.Sc thesis (Slaughter, 2004).

[1] Sub-chronic refers to a 10 day test exposure duration (longer than a typical acute test of 48-96 hours) but still using lethality as an test endpoint, and not a sub-lethal test endpoint typically used in chronic tests.

4. Acute and chronic data for indigenous South African species from sources in South Africa other than 2 and 3 listed above. Acute 96 hour data were provided by EcoSun laboratories, Rand Afrikaans University (RAU) laboratories, a M.Sc thesis (O'Brien, 2004) and an honours thesis (Tyson, 1993). Data from the two theses were screened using a quality rating score shown in table 3.1 (Warne, 2001). This screening system is based on the AQUIRE method and the quality of toxicity data are classed as complete (score between 85 and 100), moderate (score between 51 and 84) or incomplete (score of 50 or less) (Warne, 2001). Data from Tyson's thesis (1993) scored a data quality rating of complete (90%) and data from O'Brien's thesis rated moderate (58-62%). Hence, the data from these theses were considered suitable for use in guideline derivation (Warne, 2001). Chronic data were provided by the CSIR Toxicity Testing laboratory in Pretoria (Slabbert, 2004).

Table 3.1: Questions and marking system for screening toxicity data (Warne, 2001), modified from the USEPA (2004)

Question	Possible Marks*
Was the duration of the exposure stated?	20 or 0
Were there appropriate controls (e.g. a solvent control if solvent are used)?	5 or 0
Were the characteristics of the test organism stated?	5 or 0
Were the chemical concentrations measured?	5 or 0
Was the type of exposure (e.g. static, flow through) stated?	5 or 0
Was the test location stated?	4 or 0
Was the grade of purity of the test chemical stated?	4 or 0
Was the type of test media used stated?	4 or 0
Was the hardness (for freshwater) or the salinity (for saltwater) measured and stated?	2 or 0
Was the alkalinity (for freshwater) or salinity (for saltwater) measured and stated?	2 or 0
Was the dissolved oxygen content of the test water measured at some stage during or after the test?	2 or 0
Was the temperature measured during the test?	2 or 0
Was the pH of the test water measured at some time during the test?	2 or 0
Was the biological endpoint clearly defined?	20 or 0
Was there a concentration-response relationship either observed or stated?	5 or 0
Was the biological effect quantified i.e. 50% effect, 25% effect?	5 or 0
Was the statistical level of significance for any statistical tests stated (for NOEC/LOEC data)? Was a valid model used to derive the LC50/EC50 values (for LC/EC data)?	4 or 0
Was the stated significance level 0.05 or less (for NOEC/LOEC data)? Was there an estimate of the variability of the LC_{50} or EC_{50} (for LC/EC data)?	4 or 0

* There are only two marks that can be awarded in answering a question - the full mark or zero

Using all the above sources of toxicity data for NaCl and Na_2SO_4, 6 SSDs were derived and used to further derive BVs. Table 3.2 summarises the data that were used to derive the 6 different SSDs, the sources of these data and whether SSDs were derived for both salts or not.

Table 3.2: ***Summary of data used to derive 6 different species sensitivity distributions (SSDs) showing sources of data used and whether or not species sensitivity distributions were derived for both NaCl and Na_2SO_4***

SSD	Data used to derive SSD	Data source/s	Data available for NaCl?	Data available for Na_2SO_4?
1	Acute data with an assessment factor of 10	AQUIRE database; EcoSun laboratories; IWR-UCEWQ* database; O'Brien, 2003; RAU laboratories; Tyson, 1993;	**Y**	**Y**
2	Acute data applied with an acute to chronic ratio	AQUIRE database; EcoSun laboratories; IWR-UCEWQ* database; O'Brien, 2003; RAU** laboratories; Tyson, 1993;	**Y**	**Y**
3	Acute data extrapolated with linear regression analysis	IWR-UCEWQ* database	**Y**	**Y**
4	Sub-chronic data extrapolated with linear regression analysis	IWR-UCEWQ* database	**Y**	**N**
5	Lowest observed effect concentration (LOEC) data	AQUIRE database; IWR-UCEWQ* database	**Y**	**N**
6	No observed effect concentration (NOEC) data	AQUIRE database; IWR-UCEWQ* database	**Y**	**N**

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3.2.1 SSD_{AF} using AF method

All available acute LC_{50} data were divided by an assessment factor of 10 (Warne, 2001). Data used in SSD_{AF} and SSD_{ACR} are summarised in tables 3.3 and 3.4 for NaCl and Na_2SO_4 respectively.

Table 3.3: ***All available acute LC_{50} data for NaCl used to derive SSD_{AF} and SSD_{ACR}. Data are listed according to taxonomic grouping and the number of data points comprising the final geometric mean for each species are also listed***

Scientific name	Common name	Taxonomic grouping	Exp duration	Geometric mean (mg/L)	No. of datapt's	Data source
Tubifex tubifex	Tubificid worm	Annelid	24-96 h	9973	3	AQUIRE
Nais variabilis	Oligochaete	Annelid	48 h	2569	1	AQUIRE
Erpobdella punctata	Red leech	Annelid	48-96 h	7500	3	AQUIRE
Epischura baikalensis	Copepod	Crustacean	24 h	6	1	AQUIRE
Streptocephalus rubricaudatus	Fairy shrimp	Crustacean	24 h	4601	2	AQUIRE
Ceriodaphnia dubia	Water flea	Crustacean	24-48 h	1540	5	AQUIRE
Daphnia pulex	Water flea	Crustacean	24-48 h	2750	17	AQUIRE/IWR-UCEWQ/O'Brien 2004
Lymnaea sp.	Pond snail	Crustacean	24-48 h	3400	2	AQUIRE
Daphnia magna	Water flea	Crustacean	24-96 h	3084	24	AQUIRE
Caridina nilotica	Freshwater shrimp	Crustacean	48-96 h	6349	6	IWR-UCEWQ
Asellus communis	Aquatic sowbug	Crustacean	72-96 h	6487	4	AQUIRE
Leptodora kindtii	Water flea	Crustacean	96 h	3700	1	AQUIRE
Lirceus fontinalis	Aquatic sowbug	Crustacean	96 h	3688	2	AQUIRE
Paramelita nigroculus	Amphipod	Crustacean	96 h	13449	1	Tyson, 1993
Carassius carassius	Crucian carp	Fish	24 h	13750	1	AQUIRE
Poecilia reticulata	Guppy	Fish	24 h	20000	1	AQUIRE
Stizostedion lucioperca	Pikeperch	Fish	24 h	5000	1	AQUIRE
Carassius auratus	Goldfish	Fish	24-96 h	8127	0	AQUIRE
Gambusia affinis	Western mosquitofish	Fish	24-96 h	17915	3	AQUIRE
Lepomis macrochirus	Bluegill	Fish	24-96 h	7390	6	AQUIRE
Morone saxatilis	Striped bass	Fish	24-96 h	2340	8	AQUIRE
Pimephales promelas	Fathead minnow	Fish	24-96 h	7643	53	AQUIRE
Poecilia latipinna	Sailfin molly	Fish	25-48 h	17633	2	AQUIRE
Oncorhynchus mykiss	Rainbow/donaldson trout	Fish	72-96 h	5151	5	AQUIRE
Anguilla rostrata	American eel	Fish	96 h	19584	2	AQUIRE
Barbus trimaculatas	3-spot barb	Fish	96 h	5576	3	EcoSun laboratories
Clarias gariepinus	Sharptooth catfish	Fish	96 h	5530	1	O'Brien, 2003
Gambusia holbrooki	Eastern mosquitofish	Fish	96 h	11540	1	AQUIRE
Oreochromis mosssambicus	Mozambique Tilapia	Fish	96 h	1111	1	O'Brien, 2003
Tilapia sparmani	Banded tilapia	Fish	96 h	12867	5	EcoSun laboratories
Baetis tricaudatus	Mayfly	Insect	24-48 h	6706	14	AQUIRE
Argia sp.	Damselfly	Insect	48-96 h	25677	6	AQUIRE
Chimarra sp.	Caddisfly	Insect	96 h	5650	1	AQUIRE

Table 3.3: (Continued)

Scientific name	Common name	Taxonomic grouping	Exp duration	Geomean (mg/L)	No. of datapt's	Data source
Begusia sp.	Flatworm	Flatworm	96 h	5600	1	IWR-UCEWQ
Chironomus attenuatus	Midge	Insect	24-48 h	9095	3	AQUIRE
Culex sp.	Mosquito	Insect	24-48 h	10349	2	AQUIRE
Cricotopus trifasciatus	Midge	Insect	48 h	62210	1	AQUIRE
Hydropsyche sp.	Caddisfly	Insect	48 h	9000	1	AQUIRE
Hydroptila angusta	Caddisfly	Insect	48 h	6621	1	AQUIRE
Stenonema rubrum	Mayfly	Insect	48 h	2500	1	AQUIRE
Adenoplebia sylvatica	Mayfly	Insects	96 h	6539	2	IWR-UCEWQ
Afronurus barnardi	Mayfly	Insects	96 h	4957	2	IWR-UCEWQ
Afronurus peringueyi	Mayfly	Insects	96 h	5835	3	IWR-UCEWQ
Afroptilum sudafricanum	Mayfly	Insects	96 h	7908	1	IWR-UCEWQ
Baetis harrisoni	Mayfly	Insects	96 h	1631	2	IWR-UCEWQ
Caenid sp.1	Mayfly	Insects	96 h	4800	1	IWR-UCEWQ
Cloeon virgilae	Mayfly	Insects	96 h	4682	1	IWR-UCEWQ
Coenagrionidae	Damselfly	Insects	96 h	22126	2	IWR-UCEWQ
Demoreptus natalensis	Mayfly	Insects	96 h	3800	1	IWR-UCEWQ
Euthraulus elegans	Mayfly	Insects	96 h	6938	10	IWR-UCEWQ
Leptoceris sp.	Cassed caddisfly	Insects	96 h	6826	2	IWR-UCEWQ
Oligoneuropsis lawrencei	Mayfly	Insects	96 h	4816	1	IWR-UCEWQ
Plea pullula	True bug	Insects	96 h	6740	1	IWR-UCEWQ
Suragina sp.	Snipe fly	Insects	96 h	18606	1	IWR-UCEWQ
Tricorythus discolor	Mayfly	Insects	96 h	5038	8	IWR-UCEWQ
Physa gyrina	Pouch snail	Mollusc	24 h	5060	1	AQUIRE
Physa heterostropha	Pond/pneumonate snail	Mollusc	24-96 h	5714	13	AQUIRE
Helisoma campanulatum	Ramshorn snail	Mollusc	48-96 h	6571	3	AQUIRE
Gyraulus circumstriatus	Flatly coiled gyraulus	Mollusc	72-96 h	3441	2	AQUIRE
Burnupia stenochorias	Limpet	Mollusc	96 h	3899	1	IWR-UCEWQ
Caenorhabditis elegans	Nematode	Nematode	24-48 h	21295	9	AQUIRE
Limnodrilus hoffmeisteri	Tubificid worm, Oligochaete	Oligochaete	48-96 h	6642	3	AQUIRE
Polycelis nigra	Planarian	Planarian	48 h	6966	2	AQUIRE
Navicula seminulum	Diatom	Plant	96 h	2430	1	AQUIRE
Rana breviceps	Frog	Vertebrate	24-76 h	2987	35	AQUIRE

Table 3.4: ***All available acute LC_{50} data for Na_2SO_4 used to derive SSD_{AF} and SSD_{ACR}. Data are listed according to taxonomic grouping and the number of data points comprising the final geometric mean for each species are also listed***

Scientific name	Common name	Taxonomic grouping	Exp duration	Geometric mean (mg/L)	No. of datapoints	Data source
Amphipoda	Scud order	Crustacean	24-96 h	1196	4	AQUIRE
Caridina nilotica	Freshwater shrimp	Crustacean	96 h	6148	7	IWR/UCEWQ
Ceriodaphnia dubia	Water flea	Crustacean	24-48 h	3266	3	AQUIRE
Daphnia magna	Water flea	Crustacean	24-96 h	3712	12	AQUIRE
Daphnia pulex	Water flea	Crustacean	96 h	1861	4	IWR/UCEWQ
Clarius gariepinus	Sharptooth catfish	Fish	96 h	240	1	RAU laboratories
Gambusia affinis	Western mosquitofish	Fish	24-96 h	5151	6	AQUIRE
Lepomis macrochirus	Bluegill	Fish	24-96 h	9841	8	AQUIRE
Morone saxatilis	Striped bass	Fish	24 -96 h	420	16	AQUIRE
Oncorhynchus mykiss	Rainbow/donaldson trout	Fish	24 h	704	1	AQUIRE
Poecilia latipinna	Sailfin molly	Fish	24-48 h	17904	2	AQUIRE
Tilapia sparmani	Banded tilapia	Fish	96 h	14218	1	EcoSun laboratories
Afronurus barnardi	Mayfly	Insect	96 h	5979	1	IWR/UCEWQ
Baetis harrisoni	Mayfly	Insect	96 h	3620	1	IWR/UCEWQ
Cloeon virgilae	Mayfly	Insect	96 h	3369	1	IWR/UCEWQ
Coenagrionidae	Damselfly	Insect	96 h	26461	3	IWR/UCEWQ
Culex sp.	Mosquito	Insect	24-48 h	12353	2	AQUIRE
Euthraulus elegans	Mayfly	Insect	96 h	8560	2	IWR/UCEWQ
Leptocerid sp.	Cased caddisfly	Insect	96 h	10501	2	IWR/UCEWQ
Oligoneuropsis lawrencei	Mayfly	Insect	96 h	704	1	IWR/UCEWQ
Plea pullula	True bug	Insect	96 h	9355	1	IWR/UCEWQ
Tricorythus discolor	Mayfly	Insect	96 h	2818	5	IWR/UCEWQ
Burnupia stenochorias	Limpet	Mollusca	96 h	4914	2	IWR/UCEWQ
Lymnaea sp.	Pond snail	Mollusca	24-96 h	4864	4	AQUIRE
Begusia sp.	Flatworm	Flatworm	96 h	8153	1	IWR/UCEWQ
Polycelis nigra	Planarian	Planarian	48 h	6818	1	AQUIRE
Navicula seminulum	Diatom	Plantae (kingdom)	96 h	1900	1	AQUIRE

3.2.2 SSD_{ACR} using the ACR approach

An acute to chronic ratio (ACR) is the ratio of acute toxicity data to chronic toxicity data for a particular toxicant. It is calculated by dividing acute toxicity endpoints by chronic endpoints. Typically they must be calculated for the same species and as recommended by Warne (2001), have been presented in the same scientific paper or at least determined in the same laboratory. The application of ACRs to acute data is an alternative to the AF method. Chronic data used in deriving the ACRs were defined as having an experimental duration of greater than 96 hours. Chronic data used included LC_{50}s, LETCs (Lethal threshold concentration), LOECs (lowest observed effect concentration), MATC[1]s (maximum acceptable toxicant concentration), NOECs (no observed effect concentration), NR-ZERO[2] (Zero mortality) and NR-LETH[3] (lethal).

ACRs were calculated where there were acute and chronic data for the same species sourced from either the same paper or same laboratory. In some instances, more than one set of acute and chronic data existed from the same paper or laboratory for the same species. Once the ACRs for each species for each salt had been calculated, the geometric mean of all the ACR values for each salt was calculated. The geometric mean ACR value was then used as the ACR and applied to all acute data. All acute data, used to derive SSD_{ACR} with the ACR applied are summarised in tables 3.5 and 3.6 for NaCl and Na_2SO_4 respectively.

[1] MATC or Maximum Acceptable Toxicant Concentration is the hypothetical threshold concentration that is the geometric mean between the NOEC and LOEC concentration.

[2] NR-LETH: 100% mortality or 0% survival of organisms. No statistically derived endpoint reported.

[3] NR-ZERO: 0% mortality or 100% survival of organisms. No statistically derived endpoint reported.

Table 3.5: Acute and chronic data for NaCl used to derive acute to chronic ratios which were applied to acute data used in deriving SSD_{ACR} for NaCl (acute to chronic ratios were calculated by dividing acute geometric means by chronic geometric means for the same species tested in the same laboratory or where data were reported in the same paper for the same species)

Scientific name	Common name	Taxonomic grouping	Acute geometric mean (mg/L)	No. of data-points	Chronic geometric mean (mg/L)	No. of data-points	ACR	Data source
Asellus communis	Aquatic sowbug	Crustacean	6487	4	6703	3	0.97	AQUIRE
Carassius auratus	Goldfish	Fish	8142	54	7239	17	1.12	AQUIRE
Carassius auratus	Goldfish	Fish	7341	1	7322	1	1.00	AQUIRE
Chironomus attenuatus	Midge	Insects	9095	3	600	2	15.16	AQUIRE
Daphnia magna	Water flea	Crustacean	4477	2	3114	1	1.44	AQUIRE
Daphnia pulex	Water flea	Crustacean	2117	2	400	12	5.30	AQUIRE
Erpobdella punctata	Red leech	Annelid	7500	3	7500	1	1.00	AQUIRE
Gyraulus circumstriatus	Flatly coiled gyraulus	Mollusc	3441	2	3200	1	1.08	AQUIRE
Helisoma campanulatum	Ramshorn snail	Mollusc	6571	3	6150	1	1.07	AQUIRE
Limnodrilus hoffmeisteri	Tubificid worm, Oligochaete	Annelid	6642	3	5898	4	1.13	AQUIRE
Oncorhynchus mykiss	Rainbow/donaldson trout	Fish	6743	2	2029	7	3.32	AQUIRE
Oncorhynchus mykiss	Rainbow/donaldson trout	Fish	6743	2	1233	5	5.47	AQUIRE
Physa heterostropha	Pond/ pneumonate snail	Mollusc	5714	13	5100	1	1.12	AQUIRE
Pimephales promelas	Fathead minnow	Fish	7729	48	7638	13	1.01	AQUIRE
Pimephales promelas	Fathead minnow	Fish	7650	1	7650	1	1.00	AQUIRE
Pimephales promelas	Fathead minnow	Fish	6930	2	650	6	10.66	AQUIRE
Rana breviceps	Frog	Vertebrate	2987	35	1924	16	1.55	AQUIRE
Afronurus barnardi	Mayfly	Insects	4957	2	3156	1	1.57	IWR-UCEWQ
Afronurus peringueyi	Mayfly	Insects	5835	2	2922	3	2.00	IWR-UCEWQ
Caridina nilotica	Freshwater shrimp	Crustacean	6349	6	1900	1	3.34	IWR-UCEWQ
Euthraulus elegans	Mayfly	Insects	6938	10	4446	10	1.56	IWR-UCEWQ
Tricorythus discolor	Mayfly	Insects	5038	9	1966	13	2.56	IWR-UCEWQ

Table 3.6: ***Acute and chronic data for Na_2SO_4 used to derive acute to chronic ratios which were applied to acute data used in deriving SSD_{ACR} for Na_2SO_4 (acute to chronic ratios were calculated by dividing acute geometric means by chronic geometric means for the same species tested in the same laboratory or where data were reported in the same paper for the same species)***

Taxonomic grouping	Acute geomean	No. of data-points	Chronic geomean (mg/L)	No. of data-points	ACR	Data source
Crustacean	6148.07	6	1900.00	3	3.42	IWR/UCEWQ
Crustacean	3711.61	12	4547.00	1	0.82	AQUIRE
Fish	5150.89	6	2653.30	2	1.94	AQUIRE
Insect	2818.02	6	1263.12	5	2.23	IWR/UCEWQ
Flatworm	8153.00	1	6932.60	1	1.18	IWR/UCEWQ

3.2.3 SSD_{LRAA} and SSD_{LRAC} - Acute and sub-chronic data extrapolated using linear regression analysis

The IWR-UCEWQ database provided acute and sub-chronic data for which there were concentration-response data over time. These data were used to extrapolate to predicted chronic values using two-step linear regression analysis (LRA) (Mayer *et al*., 1994*)*. The following criteria were set for selection of data for analyses (Slaughter, 2004):

- Acute data with greater than 15% control mortality were excluded.
- Sub-chronic data with greater than 20% control mortality were excluded.
- Each experiment required a minimum of five concentrations.
- Each dataset had to show a trend of stabilising mortality data.

Acute and sub-chronic data were used to extrapolate the following LC values: $LC_{0.01}$, $LC_{0.1}$, LC_1, LC_5, and a LC_{10}. Lee *et al*. (1997) and Mayer *et al*. (1994) equate a MATC to an extrapolated LC value between the $LC_{0.01}$ and LC_{10}. The MATC is the maximum acceptable toxicant concentration and is the geometric mean between the NOEC and LOEC concentration. The LC value for which the most chronic values could be extrapolated was the LC_{10}, hence LC_{10}s values were used to derive SSD_{LRAA} and SSD_{LRAC}. LC_{10} values extrapolated from acute data were used to derive SSD_{LRAA} for NaCl and Na_2SO_4 and are summarised in table 3.7. LC_{10} values extrapolated from sub-

chronic data were used to derive SSD_{LRAC} for NaCl and Na_2SO_4 and are summarised in table 3.8.

Table 3.7: ***LC_{10} values extrapolated from acute toxicity data from the IWR-UCEWQ database. LC_{10} values were used to derive SSD_{LRAA} for NaCl and Na_2SO_4***

	Species	Common name	LC_{10} (mg/L)
NaCl	*Leptoceris* sp.	Caddisfly	2940
	Coenagrionidae	Damselfly	8003
	Caridina nilotica	Freshwater shrimp	3511
	Burnupia stenochorias	Limpet	1168
	Euthraulus elegans	Mayfly	320
Na_2SO_4	*Leptoceris* sp.	Caddisfly	4043
	Coenagrionidae	Damselfly	11151
	Caridina nilotica	Freshwater shrimp	4288
	Burnupia stenochorias	Limpet	3046
	Adenophlebia auriculata	Mayfly	4632
	Adenophlebia sudafricanum	Mayfly	1876
	Afronurus barnardi	Mayfly	851
	Cloeon virgilae	Mayfly	937

Table 3.8: ***LC_{10} values extrapolated from sub-chronic toxicity data from the IWR-UCEWQ database. LC_{10} values were used to derive SSD_{LRAC} for NaCl and Na_2SO_4***

	Species	Common name	LC_{10} (mg/L)
NaCl	*Adenophlebia auriculata*	Mayfly	23
	Afronurus barnardi	Mayfly	224
	Afronurus peringueyi	Mayfly	1247
	Baetid sp.	Mayfly	679
	Euthraulus elegans	Mayfly	1642
	Tricorythus elegans	Mayfly	353
Na_2SO_4	*Caridina nilotica*	Freshwater shrimp	3817
	Bugesia sp.	Flatworm	7042
	Tricorythis discolor	Mayfly	1219

3.2.4 SSD_{LOEC} and SSD_{NOEC} – LOEC and NOEC data

LOEC and NOEC data were used to derive SSD_{LOEC} and SSD_{NOEC} respectively. These data were sourced mostly from the USEPA AQUIRE database (USEPA, 2002). No LOEC and NOEC data were available for Na_2SO_4, hence tables 3.9 and 3.10 summarise LOEC and NOEC data used to derive SSD_{LOEC} and SSD_{NOEC} respectively for NaCl.

Table 3.9: Lowest Observed Effect Concentration data for NaCl used to derive SSD_{LOEC} showing taxonomic groupings of the data, the number of data points compiling the geomean and the data sources

Scientific name	Common name	Taxonomic grouping	Duration	Geometric mean (mg/L)	No. of data-points	Data source
Caridina nilotica	Freshwater shrimp	Crustacean	80 d	2700.00	1	IWR-UCEWQ
Daphnia pulex	Water flea	Crustacean	21 d	441.00	3	AQUIRE
Oncorhynchus mykiss	Rainbow/donaldson trout	Fish	90 d	1911.09	3	AQUIRE
Pimephales promelas	Fathead minnow	Fish	7 d	5120.32	7	AQUIRE
Stenonema modestum	Mayfly	Insect	14 d	5.03	8	AQUIRE
Chlorella vulgaris	Green algae	Plant	3 - 4 mo	680.00	1	AQUIRE
Selenastrum capricornutum	Green algae	Plant	91 h	566.00	1	IWR-UCEWQ

Table 3.10: No Observed Effect Concentration data for NaCl used to derive SSD_{NOEC} showing taxonomic groupings of the data, the number of data points compiling the geomean and the data sources

Scientific name	Common name	Taxonomic grouping	Duration	Geometric mean (mg/L)	No. of data-points	Data source
Caridina nilotica	Freshwater shrimp	Crustacean	80 d	1900.00	1	IWR-UCEWQ
Ceriodaphnia dubia	Water flea	Crustacean	7 d	1500.00	1	AQUIRE
Daphnia pulex	Water flea	Crustacean	21 d	314.00	3	AQUIRE
Oncorhynchus mykiss	Rainbow/donaldson trout	Fish	90 d	933.33	3	AQUIRE
Pimephales promelas	Fathead minnow	Fish	7 d	3233.71	13	AQUIRE
Stenonema modestum	Mayfly	Insect	14 d	3.47	8	AQUIRE
Chlorella vulgaris	Green algae	Plant	3 - 4 mo	590.00	1	AQUIRE

Table 3.11 summarises the levels of protection recommended to equate to the various ecological Reserve categories for South Africa. These recommendations have been sourced from the pilot guidelines for selected organic toxicants in South Africa (Warne *et al*., draft report) and are adopted in this study to relate the derived PC values for the 6 sets of SSDs, to South Africa's ecological Reserve categories, i.e. excellent, good, fair and poor (table 3.11). Hence, PC 95s, 90s and 80s were calculated for each SSD as well as PC 99s and PC 85s.

Table 3.11: Levels of protection recommended to equate to the various ecological Reserve categories of South African waters (Warne et al., draft report)

Alternate class descriptors		Level of protection
Excellent (natural)	A	PC>95
Good	B	PC>90
	C	
Fair		PC>80
	D	
Poor	E	PC<80

3.3 Results

For NaCl, 65 datapoints were used to derive SSD_{AF} and SSD_{ACR}, of which 42 (66%) were provided by the AQUIRE database and 19 (29%) by the IWR-UCEWQ database. The remainder of the data were provided by O'Brien (2004), Tyson (1993) and EcoSun laboratories (table 3.3). The data were dominated by insects (37%) of which 22% of the data were represented by South African mayfly species (table 3.12). Fish tolerance data constituted the second largest proportion of the data at 25% followed by crustaceans (17%), mollusc (8%) and an annelid (6%). There were 2 data points for flatworms and one each for a non-fish vertebrate (frog), nematode and plant.

For Na_2SO_4, 27 datapoints were used to derive SSD_{AF} and SSD_{ACR}, of which 12 (44%) were provided by the AQUIRE database and 13 (48%) by the IWR-UCEWQ database. The remainder of the data were provided by RAU and EcoSun laboratories (table 3.4). The data were also dominated by insects (37%) of which 22% of the data were represented by South African mayfly species (table 3.13). Fish tolerance data constituted the second largest proportion of the data at 26% followed by crustaceans (19%), 2 molluscs, 2 flatworms and 1 plant datum.

For NaCl, few time-series data from the IWR-UCEWQ database were available for use in extrapolations using Linear Regression Analyses (LRA) for SSD_{LRAA} (n=5). Insects continued to dominate available data however, the number of taxonomic groups represented decreased as the amount of available data decreased. A SSD_{LRAC} could be derived for NaCl, however the sample size was small (n=6) and the data were only

represented by insects. Few LOEC and NOEC data were available for deriving SSD_{LOEC} and SSD_{NOEC} (n=7 and n=7 respectively).

For Na_2SO_4, few time-series data were also available from the IWR-UCEWQ database for use in extrapolations using LRA for SSD_{LRAA} for (n=8). Insects also dominated available data. Only one Na_2SO_4 data point was available to derive SSD_{LRAC} and no LOEC and NOEC data were available to derive SSD_{LOEC} and SSD_{NOEC}, hence SSD_{LRAC}, SSD_{LOEC} and SSD_{NOEC} for Na_2SO_4 could not be derived.

Table 3.12: Taxonomic breakdown of sample sizes for NaCl data used to derive species sensitivity distributions

	SSD_{AF} and SSD_{ACR}		**SSD_{LRAA}**		**SSD_{LRAC}**	**SSD_{LOEC}**	**SSD_{NOEC}**
PC value	**Acute**	**% of**	**Acute+ LRA**	**% of**	**Sub-chronic +LRA**	**LOEC**	**NOEC**
	n=65	**total**	**n=5**	**total**	**n=6**	**n=7**	**n=7**
annelid	**4**	6					
crustacean	**11**	17	**1**	**20**		**2**	**3**
fish	**16**	25				**2**	**2**
insect	**24**	37	**3**	**60**	**6**	**1**	**1**
(mayflies)*	(14)	(22)	(1)		(6)	(1)	(1)
mollusc	**5**	8	**1**	**20**			
flatworm	**2**	3					
vertebrate	**1**	2					
nematode	**1**	2					
plant	**1**	2				**2**	**1**

* Mayfly data were included in the total dataset for insects but are shown separately in brackets showing the large proportion of insect data dominated by mayfly data

Table 3.13: Taxonomic breakdown of sample sizes for Na_2SO_4 data used to derive species sensitivity distributions

	SSD_{AF} and SSD_{ACR}		SSD_{LRAA}	
PC value	**Acute n=27**	**% of total**	**Acute+ LRA n=8**	**% of total**
crustacean	5	19	1	13
fish	7	26		
insect	10	37	6	75
(mayflies)*	(6)	(22)	(4)	(50)
mollusc	2	7	1	13
flatworm	2	7		
plant	1	4		

* Mayfly data were included in the total data for insects but are shown separately in brackets showing the large proportion of insect data dominated by mayfly data

Tables 3.14 and 3.15 summarise the PC values derived for all the SSDs for both salts respectively. The PC 99, 90, 85 and 80 are reported and compared with the BVs proposed by Jooste and Rossouw (2002) (referred to as SSD_J) and Palmer *et al*. (2004c) (referred to as SSD_P). SSD_{ACR} provides the highest PC values, followed by SSD_{LRAA}, SSD_P and SSD_{AF}. The most conservative values are provided by SSD_{LRAC}, SSD_{LOEC} and SSD_J.

Table 3.14: Comparative summary of protective concentration values for NaCl (All values reported in mg/L)

PC value	SSD_{AF} Acute/10 n=65	SSD_{ACR} Acute/1.9 n=64	SSD_{LRAA} Acute +LRA n=5	SSD_{LRAC} Sub-chronic n=6	SSD_{LOEC} LOEC n=7	SSD_{NOEC} NOEC n=7	Ecological category	SSD_J Jooste & Rossouw (2002)	SSD_P Palmer et al. (2004c)
99	42	212	28	2	0.2	0.5			17.6
95	**120**	**617**	**179**	**19**	**8**	**10**	**N**	**45**	**146**
90	**190**	**982**	**398**	**53**	**34**	**39**	**G**	**217**	**365**
85	249	1294	636	96	83	85			
80	**303**	**1582**	**887**	**148**	**155**	**148**	**F**	**389**	**900**

Table 3.15: Comparative summary of protective concentration values for Na_2SO_4 (all values reported in mg/L

PC value	SSD_{AF} Acute/10 n=27	SSD_{ACR} Acute/1.7 n=27	SSD_{LRAA} Acute +LRA n=8	SSD_{LRAC} Sub-chronic +LRA n=1	SSD_{LOEC} LOEC	SSD_{NOEC} NOEC	Ecological category	SSD_J Jooste & Rossouw (2002)	SSD_P Palmer et al. (2004c)
99	7	41	220	N/A	N/A	N/A			155
95	**40**	**234**	**614**	N/A	N/A	N/A	**N**	**20**	**462**
90	**84**	**497**	**959**	N/A	N/A	N/A	**G**	**36**	**800**
95	131	773	1251	N/A	N/A	N/A			
80	**180**	**1058**	**1518**	N/A	N/A	N/A	**F**	**51**	**1540**

3.4 Discussion

Although the SSD approach seems to provide a consistent, objective method for deriving guidelines, the variety of data which can be selected will influence the final numerical values. Figures 3.1 and 3.2 illustrate the SSDs generated in order of increasing PC values for NaCl and Na_2SO_4 respectively. These figures are based on **general** increasing trends of PC values (a few exceptions exist, for example, SSD_{LOEC} for NaCl generally had the lowest PC values with the exception of the PC 80 which was higher than the PC 80 for SSD_{LRAC} and SSD_{NOEC}). Jooste and Rossouw (2002) acknowledge that their approach may consistently overestimate the toxicity of saline fresh waters and their values are likely to be conservative. Hence, it is possible that the values generated by SSD_{AF}, SSD_{LRAC}, SSD_{LOEC} and SSD_{NOEC}, which all generated PC values less than those proposed by Jooste and Rossouw (2002), might also be conservative. This could have implications for industry and agriculture, i.e. if guidelines are over-protective, the suggested guideline value might be unrealistic to achieve and/or economically not feasible. In the process of deriving guidelines, a balance needs to be sought between a guideline being over-protective and not allowing development, and being under-protective and compromising ecosystem health and long term sustainability of the ecosystem. These values will have to be applied in test case scenarios (see chapter 4).

Increasing PC values ↓

SSD	Type of data/method used to generate SSD
SSD_{LOEC}	LOEC
SSD_{NOEC}	NOEC
SSD_{LRAC}	Sub-chronic data + LRA
SSD_{AF}	Acute + AF of 10
SSD_{J}	Jooste & Rossouw (2002)
SSD_{P}	Palmer et al. (2004c)
SSD_{LRAA}	Acute + LRA
SSD_{ACR}	Acute + ACR of 1.9

Figure 3.1: ***Species sensitivity distributions listed in order of general increasing PC Values for NaCl, based on results presented in table 3.14***

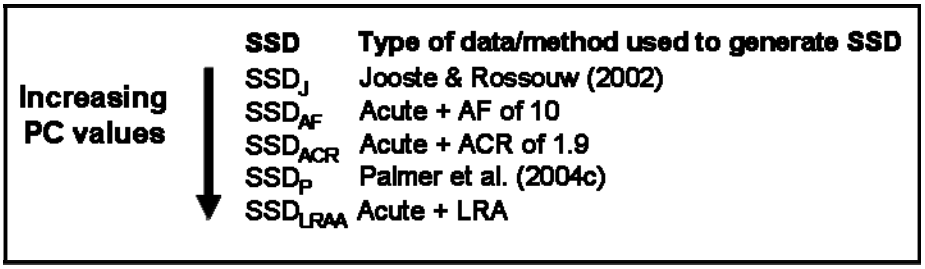

Figure 3.2: ***Species sensitivity distributions listed in order of general increasing PC Values for Na_2SO_4 based on results presented in table 3.15***

The SSD approach is useful for water resource managers that are interested in estimating safe thresholds an effort to derive WQGs. They allow water resource managers to account for a range of taxa for which toxicity data are only available for a small proportion of those that might be exposed to the chemical (Grist *et al*., 2002; Pennington, 2003). Ideally WQGs should be derived using chronic data as these would most confidently provide protection for aquatic ecosystems. Chronic tests more accurately reflect toxic effects representative of what would happen in a real aquatic environment and can include long-term exposure effects such as growth inhibition or decreased reproduction rates.

However, toxicity datasets are commonly dominated by acute data (Pennington, 2003) as was evident in this study. Acute data constituted the most number of data. A large proportion (46%) of these acute data were provided by the results of chapter 2. Acute data are more prevalent than chronic data as acute experiments are less complex, less expensive and more readily conducted in laboratories due to their short-term exposure times and endpoints which are more easily observed and measured (e.g. mortality) compared to those with chronic endpoints such as reproduction and growth (Cooney, 1995).

Forbes and Calow (2002) caution that attention needs to be given to the source and composition of input data for a SSD. Confidence in output data is strongly dependent on the number of test results entered in the SSD (Aldenberg and Jaworska, 2000; Grist *et al*., 2002; Pennington, 2003), hence a high data confidence would typically be associated with SSDs derived using many acute datasets, and a low confidence associated with SSDs derived using fewer chronic datasets (figure 3.3). Contrastingly, SSDs are used differently in the Australian and New Zealand method. SSDs are used

for the purpose of deriving WQGs and hence have different data requirements and different confidences associated with them. Chronic data are used to derive high reliability TVs where NOEC data exist for 5 or more species, representing 4 or more taxonomic groups (Warne, 2001). According to this method, the results derived from SSD_{NOEC} could be used to derive high reliability TVs (table 3.12) as NOEC NaCl data exists for 7 species from 4 taxonomic groups (crustacean, fish, insect and plant). Solomon *et al*. (1996) and Wheeler *et al*. (2002) suggest a minimum data requirement of 10 species for use in a SSD and hence, the results generated from SSD_{LRAA}, SSD_{LRAC}, SSD_{LOEC} and SSD_{NOEC} for NaCl and SSD_{LRAA} for Na_2SO_4 would be associated with a low confidence in the generated PC values. The BurrliOz programme gives a warning for SSDs generated using 8 or fewer data points for the results to be interpreted with caution. Consequently, the BurrliOz warning would also apply to SSD_{LRAA}, SSD_{LRAC}, SSD_{LOEC} and SSD_{NOEC} for NaCl and to SSD_{LRAA} for Na_2SO_4.

One of the main advantages of using the SSD approach is that small datasets can be extrapolated to provide protection for theoretically 95% of a population throughout their life cycle and so account for unknown species sensitivities (Pennington, 2003). By ensuring that the dataset used to derive the SSD represents as broad a range of species sensitivities as possible, the extrapolated SSD is more likely to accurately predict the PC 95. This becomes less feasible with smaller datasets as is shown in table 3.12 where the number of taxonomic groupings represented by each SSD for NaCl decreased from 9 for SSD_{AF} and SSD_{ACR}, to 4 taxonomic groups for SSD_{LOEC} and SSD_{NOEC}, 3 for SSD_{LRAA} and only 1 group represented by insects for SSD_{LRAC}.

The typical trade off then exists for using either chronic data or acute data: data confidence for environmental realism (or vice versa) (figure 3.3). Acute data are dominated by larger sample sizes compared to chronic data resulting in a higher confidence in the data, however, chronic data, typically characterised by fewer sample sizes and a lower confidence in data, provide a higher degree of environmental realism.

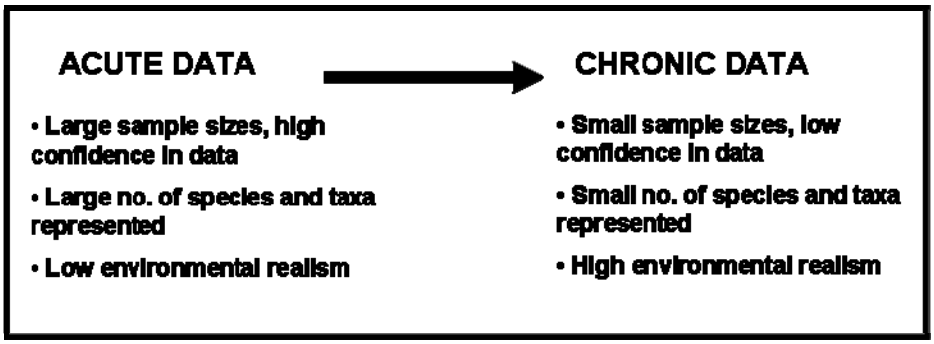

Figure 3.3: ***Diagram showing the effects on data confidence and environmental realism when using acute vs. chronic data***

In an effort to derive accurate and representative guidelines for aquatic ecosystems, despite a small available amount of chronic data, some work has focused on acute to chronic data extrapolations. One method applied in this study is that of linear regression analysis (LRA) (Mayer *et al*., 1994). Acute and sub-chronic data were extrapolated to LC_{10}s by Slaughter (2004)[1] and the LC_{10}s values used to derive SSD_{LRAA} and SSD_{LRAC}. LRA was applied separately to existing acute and sub-chronic data. Few data were suitable for extrapolation as the method is dependant on time-series data for the duration of a toxicity experiment. The few number of datasets available, reflect the lack of time-series data published in national and international data (all data extrapolated were provided by the IWR-UCEWQ database). *Authors would be making a valuable contribution to future work on extrapolations by providing comprehensive concentration-time data when reporting toxicity data in literature.* As can be expected, PC values derived using sub-chronic data were considerably lower than those derived using acute data as high LC_{50} values used for these extrapolations would have yielded higher extrapolated LC_{10} values. Interestingly, PC values derived from SSD_{LRAC} using extrapolated sub-chronic data closely compared to the PC values derived using SSD_{LOEC} and SSD_{NOEC}, i.e. using NOEC and LOEC data respectively. Sub-chronic toxicity tests with mortality as endpoints are slightly more lengthy than acute tests, but do not incur some of the complexities associated with observing chronic test endpoints such as growth and reproduction. Furthermore, the results generated from SSD_{LRAC} suggest that by focusing future toxicity tests on deriving sub-chronic endpoints and extrapolating these using LRA, these will closely compare to measured chronic endpoints and would be most reliable in a management application

[1] Slaughter (2004) has examined other extrapolation methods, however only data extrapolated with linear regression analysis were available at the time of writing this study.

such as the derivation of WQGs. Furthermore, extrapolated sub-chronic endpoints provide the most environmental realism with comparatively less laboratory effort to that of chronic tests.

CHAPTER 4: The application of recommended water quality guidelines in the Upper Olifants River catchment: a test case

4.1 Introduction

The importance of the ecological Reserve (termed “Reserve” in this chapter) and some of the key tools used in determining a Reserve for a specific river were outlined in chapter 1. Reserve determinations can be carried out at a desktop, intermediate and comprehensive level (DWAF, 2003). A comprehensive Reserve determination is the most costly and time-consuming, but provides the highest level of confidence in the established RQOs as current field-collected data are used together with historical data. Until recently, EC and TDS have been used to establish RQOs for salinity in the Reserve determination process. This is mainly due to the fact that EC and TDS are easily measured and determining individual ionic compositions involves a more complex process. Furthermore, EC and TDS data are more easily obtainable at study sites than individual ionic data. However, the need for RQOs which accommodate for individual salt toxicity is evident because salt toxicity in freshwater systems is dependant on specific ion composition of the water (Jooste and Rossouw, 2002; Mount *et al*., 1997).

Jooste and Rossouw (2002) first proposed a toxicity-based model for describing the hazards of individual salts referred to as the toxicologically important major salt (TIMS) model. The concept of the TIMS model is based on the idea that a salt will exist in solution as ions stabilised by water molecules. If two salts of relatively low toxicity are in solution, for example NaCl and Ca_2SO_4, the water chemistry has the potential for a more toxic salt to form, in this case Na_2SO_4, which could adversely affect resident organisms. This salt then becomes the major toxicant even though it may not have originally been discharged into the system (Jooste and Rossouw, 2002).

The TIMS model recognises the following salts as toxicologically important, in order of increasing toxicity: calcium sulphate ($CaSO_4$), sodium chloride (NaCl), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), sodium sulphate ($Na_2SO_{4)}$ and magnesium sulphate ($MgSO_4$). Jooste developed a model (SaltBA23.exe) (Jooste, 2004) which, given the known ionic composition in a given body of water, will predict the likely concentrations of inorganic salts to form. These concentrations can then be compared

to generic or re-calibrated site-specific benchmark values for each TIMS and management classes assigned. These benchmark values were established using toxicity data extracted from the USEPA ECOTOX database (USEPA, 2002). Two initial benchmarks were established using extracted data. Firstly, a lethality benchmark which was based on the LC_{50} projected to 2 weeks (336 hours) of exposure and was calculated as the 5^{th} percentile of the LC_{50} (336h) dataset. This formed the benchmark between the 'D' and 'E' ecological Reserve categories. Secondly, the sub-lethality benchmark which was set at the 5^{th} percentile of all sub-lethal data available. This formed the benchmark between the 'A' and 'B' ecological Reserve categories. The remaining benchmark values were derived from an interpolation of a hazard-based stressor response curve which was generated from the lethality and sub-lethality benchmarks (Palmer *et al*., 2004b).

This chapter aims to apply the different BVs derived (for NaCl and Na_2SO_4) using the different datasets from chapter 3 to a case study where a Reserve determination has already been completed and ecological Reserve categories and management classes established according to EC. An intermediate Reserve determination for water quality had already been completed for the Olifants River (DWAF, 2000b) and provided the necessary sets of water chemistry and toxicological data needed to apply the findings of chapter 3. By gathering ionic data for select sites along the Olifants River and using Jooste's salt spreadsheet, the TIMS concentrations for NaCl and Na_2SO_4 were determined. These individual salt concentrations, used to derive present state assessments, were then compared to the various BVs derived in chapter 3 and the implications for management from using different types of data are discussed.

4.2 Study area

The intermediate Reserve determination for water quality for the Olifants River is summarised in the Olifants River Ecological Requirements Assessment report (OREWRA) (DWAF, 2000b). The Olifants River catchment was sub-divided into three main study areas, mainly the Upper, Middle and Lower Olifants (figure 4.1). This chapter examines the Upper Olifants study area which is the main catchment upstream of Loskop Dam. Only the Upper Olifants catchment is used in this assessment due to time constraints. This area comprises the Upper Olifants River, Klein Olifants River and Wilge River with its tributaries downstream to Loskop Dam (figure 4.1). The headwaters of the study area are located along the Highveld Ridge in the Secunda-Bethal area. Key characteristics of the study area include:

- the presence of various weirs and impoundments of varying capacities, including the Witbank, Bronkhorstspruit, Middleburg and Premier Mine Dams upstream of Loskop Dam;
- extensive coal mining for coal-fired power stations which have both water demand and water quality implications;
- land-use is dominated by dryland and irrigation agriculture; and
- the presence of intensive farming in the form of piggeries and cattle feed-lots.

Hence, water quality concerns include:

- High sulphate and dissolved solids (TDS)
- High sulphate concentrations
- Low pH
- High concentrations of iron, manganese and aluminium as a result of mining activities.

The Wilge River catchment was largely unpolluted which probably improved inflowing water quality into Loskop Dam (DWAF, 2000b).

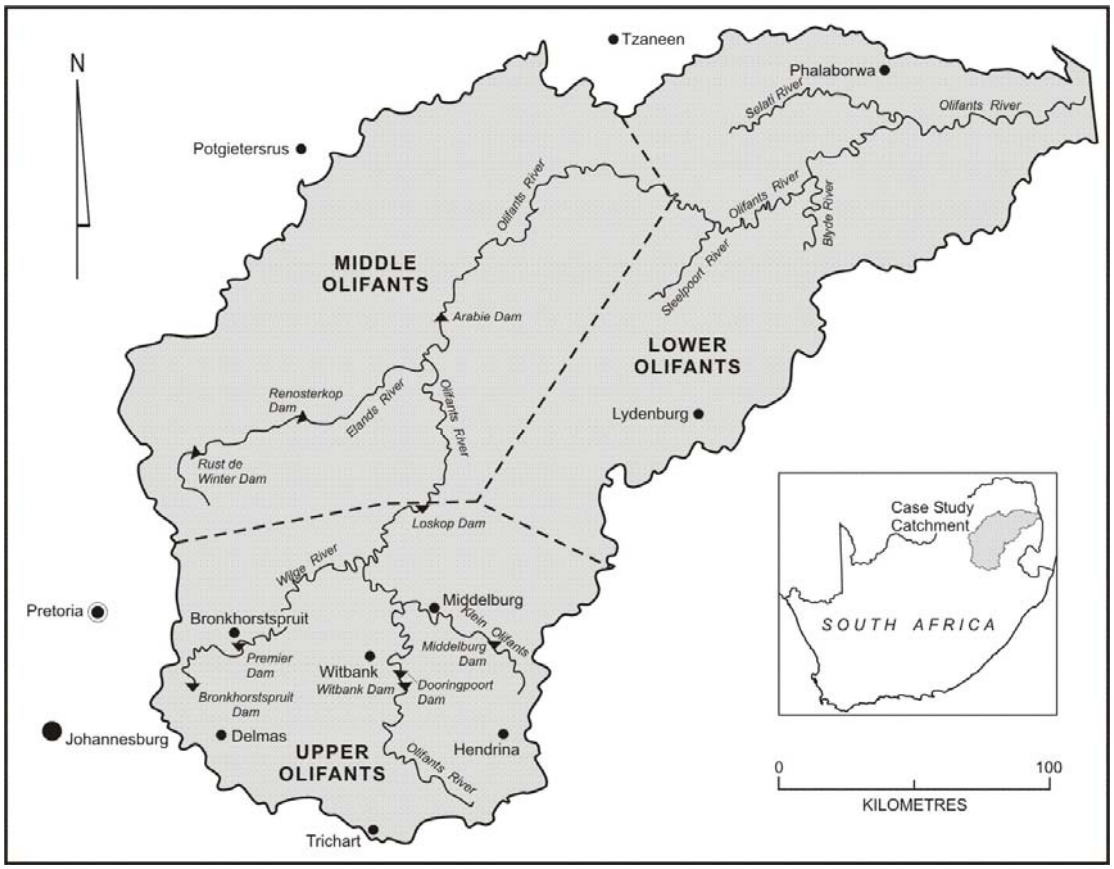

Figure 4.1: Map of the Olifants River catchment showing the Upper, middle and lower catchments (Palmer et al., 2004d)

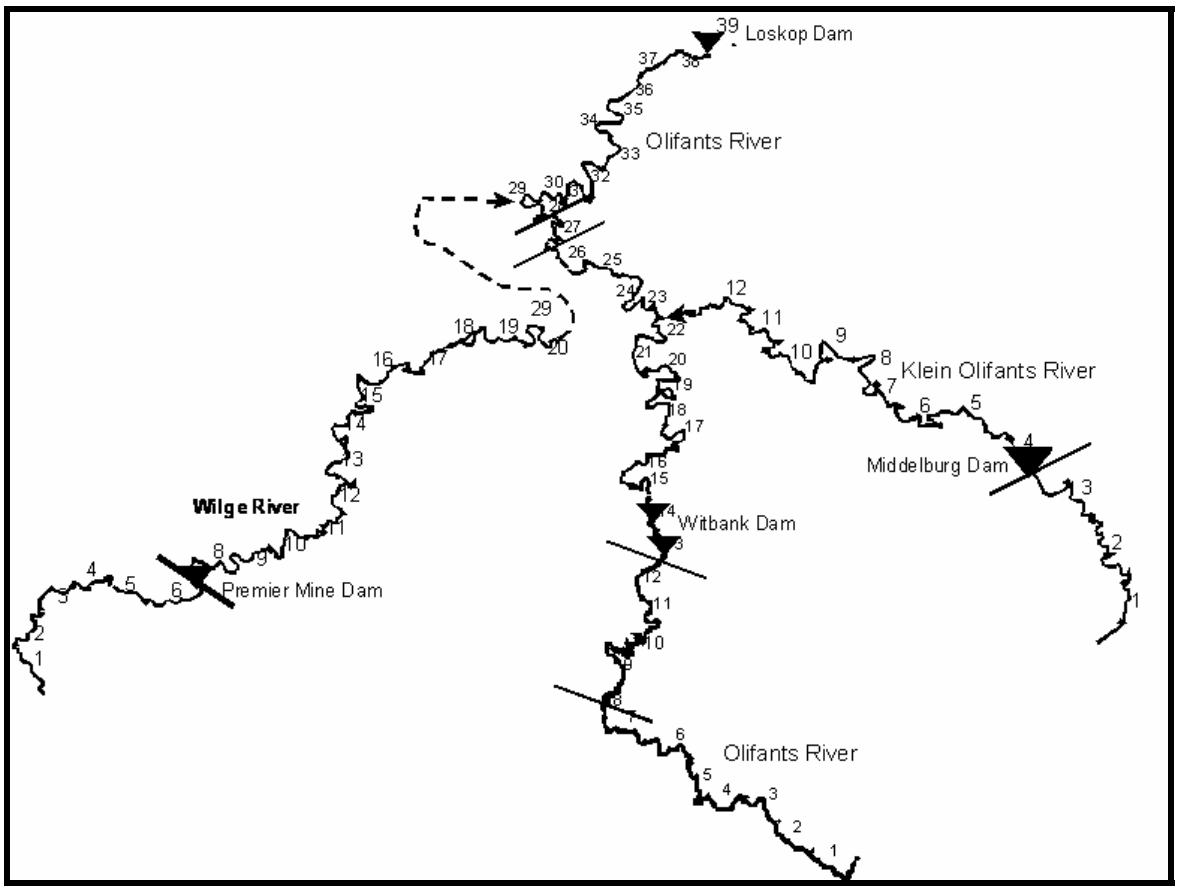

Figure 4.2: ***Map of the Upper Olifants River study area showing segment numbers referred to in table 4.1 (DWAF, 2000b)***

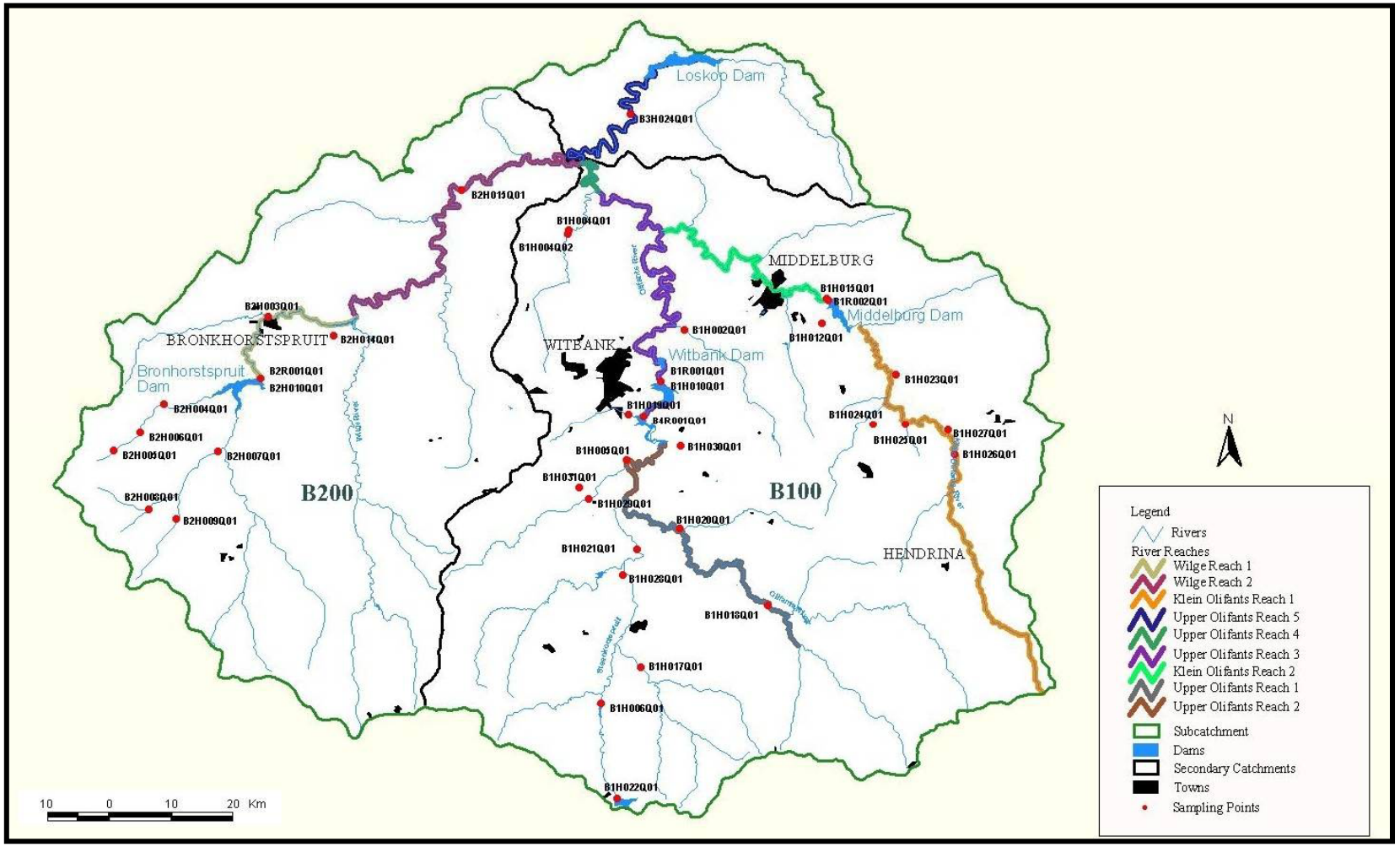

Figure 4.3: ***Map showing the location of the DWAF water quality monitoring points and the different river reaches of the Upper Olifants catchment (DWAF, 2000b)***

4.3 Methods

The Upper Olifants study area had been delineated into water quality reaches (figures 4.2 and 4.3) where reaches were significantly different from each other to warrant their own specification of a water quality Reserve and where water quality was considered to be homogenous for a particular river reach (method specified in Palmer *et al*., in press a). Reaches were divided into segments. These segments are summarised in table 4.1 with descriptions of the water quality reaches, and are illustrated in figures 4.2 and 4.3 (DWAF, 2000b). For each river reach, DWAF water quality monitoring points were selected to provide reference and present ecological state (PES) site data (where these monitoring points were available) and these monitoring points are denoted by their weir numbers (table 4.1 and figure 4.3).

Water quality data for these weirs were obtained from the DWAF Hydrological Information System (HIS) database. The exact data records used in the original Olifants study were not used as the report did not provide indication of these (DWAF, 2000b). Ionic concentration data (for Na, Mg, SO_4, Cl, K and Ca ions) were extracted from these data. Data were prepared to run through the TIMS model accordingly:

- Each set of ionic data for each weir was considered separately.
- Values recorded as below detection limits (denoted by a "<") were converted to half the detection limit value. This has been deemed statistically appropriate (Palmer *et al*., in press a).
- If there were data missing for any water quality variables for a given sample, the entire data record was excluded from the analysis in order to prevent inaccuracies when importing data to the salt model.
- Care was taken not to change any header names or order of columns as the model currently requires these to be consistent with DWAF's data format.
- The file is then saved as a 'comma delineated' file (file extension ".csv") and is ready for use in Jooste's salt spreadsheet.

Table 4.1: Water quality reaches for the Upper Olifants River (from DWAF, 2000b)

River reach delineated by segment	Description	Comments	Weir Number	
			Reference site	PES site
Olifants 1-8	Olifants River from its source to the confluence with the Steenkoolspruit	The upper reaches of the Olifants are relatively undisturbed with dryland agriculture being the main land use and some coal mining at the bottom end of the reach.	B1H006Q01	B1H018Q01
Olifants 9-13	Olifants River from the Steenskoolspruit confluence to the inflow into Witbank Dam	This reach of the Olifants is highly impacted by coal mining activities in the catchment.	B1H018Q01	B1H005Q01
Olifants 14-27	Olifants River downstream of Witbank Dam to the Klipsruit confluence	This river reach is negatively impacted by water from the Spookspruit (due to coal mining activities) and the Klein Olifants River. There are no routine DWAF monitoring stations in this reach which can be used to assess the PES.		
Olifants 28	Olifants River from the Klipspruit confluence to Wilge River confluence	This river reach is negatively impacted by the poor water quality in the Klipspruit (due to old coal mining activites). There are no routine DWAF monitoring stations in this reach which can be used to assess the PES.		
Olifants 29-37	Olifants from the Wilge River confluence to the inflow into Loskop Dam	This reach is positively impacted by the good quality water in the Wilge River. There are no routine monitoring stations in this reach which can be used to assess the PES although the water quality in Loskop Dam was used to estimate the PES.		B3R002Q01
Klein Olifants 1-4	Klein Olifants upstream of Middleburg Dam	The Klein Olifants River is highly affected by coal mining and power generation activities in its catchment.	B1H026Q01	B1H012Q01
Klein Olifants 5-12	Klein Olifants from downstream of Middleburg Dam to the confluence with the Olifants River	There are no routine DWAF monitoring stations in this reach which can be used to assess the PES and the weir downstream of Middleburg Dam was used for this purpose.	B1H026Q01	B1H015Q01
Wilge 1-6	Bronkhorstpruit from Bronkhorstpruit Dam to Premier Mine Dam	This reach is relatively unimpacted and agriculture is the main land use activity. Minor treated domestic sewage discharges at Bronkhorstpruit.	B2H007Q01	B2H003Q01
Wilge 7-20	Wilge River from Premier Mine Dam to the confluence with the Olifants River	This reach of the Wilge River is in good condition. The main land use is agriculture.	B2H014Q01	B2H015Q01

Once the data were run through the model, the data output from the model were converted to mg/L for further analysis. Reconstituted reference data[1] for NaCl and Na_2SO_4 were selected for each weir. Data were selected accordingly (Palmer *et al*., 2004b):

- A minimum of 25 samples were collected over a 1-3 year period.

[1] Reconstituted data are those individual ionic data run through Jooste's salt model to yield theoretical reconstituted concentrations of individual salts.

- Calculated 95th percentile values were compared to default Natural BVs provided by Jooste and Rossouw (2002) (table 4.2).
- If the 95th percentile values of the reference condition data were higher than the default Natural boundary values, the benchmark values were adjusted as follows:
    - the 95th percentile concentration of the reference data became the default Natural boundary value.
    - The Good boundary is moved by half the amount by which the Natural boundary was changed.

Reconstituted inorganic salt data for NaCl and Na_2SO_4, not older than 5 years, were selected to assess PES (including wet and dry season data). Using these data, the mean 95th percentiles were calculated for each salt for each weir. These values were then used to assign ecological Reserve categories (Natural (N), Good (G), Fair (F) or Poor (P)) for each salt and for each weir, by comparing the 95th percentile values against either the default benchmark values (table 4.2) or the adjusted benchmark values.

Confidences in each dataset were calculated using the G Power freeware program, Version 2 (Faul and Erdfelder, 1992) available from http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/. Confidence in a data set was classified accordingly: High confidence - G power greater than 0.8; medium confidence - G power between 0.6 and 0.8; and low confidence - less than 0.6.

The PC values which were derived using different SSDs derived in chapter 3 were used as BVs for both salts in this chapter. For each SSD for each salt, PC values were used accordingly (Warne *et al*., draft report): the PC 95 was used as the natural boundary value, PC 90 as the good boundary and PC 80 as the fair boundary (tables 4.3 and 4.4). These boundary values were then used to assign ecological Reserve categories for each weir using the 95th percentile of the PES data for each weir in the Upper Olifants. The implications for water resource managers of using different types of data to derive WQGs for salinity were examined for further discussion.

Table 4.2: Benchmark boundary values proposed by Jooste and Rossouw (2002)

Variables	Natural boundary (mg/L)	Good boundary (mg/L)	Fair boundary (mg/L)
Na_2SO_4	20	36	51
NaCl	45	217	389

Table 4.3: Comparative summary of protective concentration values derived in chapter 3 for NaCl (all values reported in mg/L)

PC value	Ecological category	SSD_{AF}	SSD_{ACR}	SSD_{LRAA}	SSD_{LRAC}	SSD_{LOEC}	SSD_{NOEC}	SSD_J	SSD_P
		Acute/10	Acute/1.9	Acute +LRA	Sub-chronic +LRA	LOEC	NOEC	Jooste & Rossouw (2002)	Palmer & Warne (2004)
		n=65	n=64	n=5	n=6	n=7	n=7		
99		42	212	28	2	0.2	0.5		17.6
95	**N**	**120**	**617**	**179**	**19**	**8**	**10**	**45**	**146**
90	**G**	**190**	**982**	**398**	**53**	**34**	**39**	**217**	**365**
85		249	1294	636	96	83	85		
80	**F**	**303**	**1582**	**887**	**148**	**155**	**148**	**389**	**900**

Table 4.4: Comparative summary of protective concentration values for Na_2SO_4 (all values reported in mg/L)

PC value	Ecological category	SSD_{AF}	SSD_{ACR}	SSD_{LRAA}	SSD_{LRAC}	SSD_{LOEC}	SSD_{NOEC}	SSD_J	SSD_P
		Acute/10	Acute/1.7	Acute +LRA				Jooste & Rossouw (2002)	Palmer et al. (2004c)
		n=27	n=27	n=8	n=1				
99		**7**	**41**	**220**	*	*	*		155
95	**N**	**40**	**234**	**614**	*	*	*	**20**	**462**
90	**G**	**84**	**497**	**959**	*	*	*	**36**	**800**
85		131	773	1251	*	*	*		
80	**F**	**180**	**1058**	**1518**	*	*	*	**51**	**1540**

*Insufficient data to derive SSD

4.4 Results

Table 4.5 summarises the results from the reconstitution of the DWAF inorganic salt data for both salts for **reference** conditions. The 95th percentile value for site B1H018Q01 (Olifants segments 9 to 13) for both salts was higher than the natural benchmark value and hence the default benchmark values were adjusted accordingly (table 4.5).

Table 4.5: Summary of results from inorganic salt data analysis for NaCl and Na_2SO_4 for reference conditions, indicating where benchmark values had to be adjusted

Weir No.	River reach	Sample size	NaCl		Na_2SO_4	
			95 ‰ (mg/L)	> 45 mg/L (natural benchmark value) ?	95 ‰ (mg/L)	> 20 mg/L (natural benchmark value)?
B1H006Q01	Olifants 1-8	99	28	No	0	No
B1H018Q01	Olifants 9-13	103	123	Yes*	25	Yes*
B1H026Q01	Klein Olifants 1-12	45	41	No	11	No
B2H007Q01	Wilge 1-6	82	19	No	0	No
B2H014Q01	Wilge 7-20	94	18	No	0	No

*95th percentile values were higher than the default Natural boundary values, hence benchmark values were recalibrated. For NaCl: N = 123, G = 257, P = 428; and Na_2SO_4: N = 25, G = 39, P = 53.

Table 4.6 summarises the results from the reconstitution of the DWAF inorganic salt data for both salts for the **present ecological state** conditions. Ecological Reserve categories for the two inorganic salts for site B1H005Q01 (Olifants segments 9-13) were assigned using adjusted benchmark values. PES conditions for NaCl were mostly categorised as natural with the exception of the upper reach of the Olifants (segments 1-8). Contrastingly, from the lower reach of the Olifants through to the lower reaches of the Klein Olifants, reaches were categorised as being in poor condition for Na_2SO_4, improving again in reaches of the Wilge River, to a natural condition in the upper reaches and to a good condition in the lower reaches of the Wilge.

Table 4.6: Summary of results from inorganic salt data analysis for NaCl and Na_2SO_4 for present ecological state conditions, showing assigned ecological Reserve categories according to Jooste and Rossouw's (2002) benchmark values

Weir No.	River reach	Sample size	NaCl		Na_2SO_4	
			95th percentile (mg/L)	Assigned health category	95th percentile (mg/L)	Assigned health category
B1H018Q01	Olifants 1-8	66	56	G	36	G
B1H005Q01	Olifants 9-13	118	79	N*	162	P*
B3R002Q01	Loskop Dam	50	29	N	54	P
B1H012Q01	Klein Olifants 1-4	208	35	N	53	P
B1H015Q01	Klein olifants 5-12	167	41	N	59	P
B2H003Q01	Wilge 1-6	83	29	N	0	N
B2H015Q01	Wilge 7-20	83	26	N	27	G

* These categories were assigned using recalibrated benchmark values

Table 4.7: Data confidence results yielded from G power calculation

			NaCl				Na_2SO_4			
	Weir No.	Sample size	Mean salt concentration (mg/L)	Standard deviation	G-Power Value	Confi-dence	Mean salt concentration (mg/L)	Standard deviation	G-Power Value	Confi-dence
Ref	B1H006Q01	99	16.41	5.09	0.62	M	0.00	0.00	-	-
	B1H018Q01	103	48.68	35.79	0.16	L	5.45	20.26	0.05	L
	B1H026Q01	45	22.35	9.51	0.20	L	79.86	45.85	0.05	L
	B2H007Q01	82	14.11	3.09	0.82	H	0.00	0.00	-	-
	B2H014Q01	94	12.51	4.62	0.45	L	0.11	1.08	0.05	L
PES	B1H018Q01	66	37.93	13.27	0.42	L	4.38	13.86	0.03	L
	B1H005Q01	118	44.48	22.55	0.33	L	79.86	45.85	0.27	L
	B3R002Q01	50	24.73	2.41	0.99	H	37.25	7.91	0.64	M
	B1H012Q01	208	27.66	4.88	1.00	H	35.87	12.27	0.84	H
	B1H015Q01	167	31.85	4.80	1.00	H	39.86	10.41	0.94	H
	B2H003Q01	83	22.92	3.95	0.96	H	0.00	0.00	-	-
	B2H015Q01	83	15.78	6.39	0.35	L	10.40	8.52	0.12	L

Data confidence results yielded from the G power calculation are summarised in table 4.7 and show that 6 datasets for both salts yielded a low confidence, 1 dataset for each salt yielded a medium confidence, and 5 and 2 datasets yielded a low confidence for NaCl and Na_2SO_4 respectively.

Table 4.8 summarises the results of assigning ecological Reserve categories to the Upper Olifants river PES sites for NaCl based on inorganic salt analyses for NaCl, using the different BVs proposed in table 4.3. Listed together with these values are the categories assigned for each river reach based on the invertebrate and TDS assessment conducted as part of the Olifants Reserve determination (DWAF, 2000b). SSD_{AF}, SSD_{ACR} and SSD_{LRAA}, and the method proposed by Palmer *et al*. (2004c) all yielded categories of 'natural' indicating very high boundary values compared to that of SSD_{LRAC}, SSD_{LOEC} and SSD_{NOEC}. SSD_{LRAC}, SSD_{LOEC} and SSD_{NOEC} yielded categories of either 'good' or 'fair'. No sites were categorised as 'natural' using SSD_{LRAC}, SSD_{LOEC} and SSD_{NOEC}. No proposed BVs yielded a category of 'poor' in contrast to the invertebrate assessment which categorised segments 9 to 13 of the Olifants as poor. Segments 1 to 4 were categorised as 'poor' according to the TDS assessment (DWAF, 2000b).

Table 4.9 summarises the results of assigning ecological categories to PES sites for Na_2SO_4, based on inorganic salt analyses and using the boundary values proposed in table 4.4. SSD_{ACR} and SSD_{LRAA} and the method proposed by Palmer *et al*. (2004c) all yielded categories of natural. Unlike the results for SSD_{AF} for NaCl which yielded categories of natural for all weir sites, SSD_{AF} for Na_2SO_4 yielded a fair condition for segments 9 to 13 of the Olifants and good condition for both reaches of the Klein Olifants. Boundary values proposed by Jooste and Rossouw (2002) yielded a natural

category for the upper reaches of the Wilge (segments 1-6) and good for the lower reaches (segments 7-20). Except for the upper reaches of the Olifants (segments 1-8) which was categorised as natural, the Olifants, segments 9-13, and both reaches of the Klein Olifants were categorised in a poor condition using the values proposed by Jooste and Rossouw (2002). This corresponds with the findings of both the invertebrate bio-assessment and the TDS assessment, where segments 9-13 of the Olifants and both reaches of the Klein Olifants were classified as either fair or poor.

Table 4.8: Results of assigning ecological Reserve categories to PES NaCl data for the Upper Olifants river reaches using different BVs derived in chapter 3 and corresponding invertebrate assessment categories

PES site weir numbers	River reach	NaCl mg/L	SSD_{AF} Acute + AF	SSD_{ACR} Acute + ACR	SSD_{LRAA} Acute + LRA	SSD_{LRAC} Sub-chronic +LRA	SSD_{LOEC} NOEC	SSD_{NOEC} LOEC	SSD_J Jooste & Rossouw (2002)	SSD_P Palmer et al. (2004c)	Bioassement category assigned for invertebrates (DWAF, 2000b): Category according to A/B/C/D/E/F classification	Bioassement category assigned for invertebrates (DWAF, 2000b): Category converted according to N/G/F/P classification to allow for comparison	TDS category (DWAF, 2000b)
B1H 018 Q01	Olifants 1-8	56	N	N	N	F	F	F	G	N	C	G/F	F
B1H 005 Q01	Olifants 9-13	79	N	N	N	F	F	F	G	N	E	P	F
B1H 012 Q01	Klein Olifants 1-4	35	N	N	N	G	G	F	N	N	D	F	P
B1H 015 Q01	Klein Olifants 5-12	41	N	N	N	G	F	F	N	N	C	G/F	F
B2H 003 Q01	Wilge 1-6	29	N	N	N	G	G	G	N	N	C	G/F	N
B2H 015 Q01	Wilge 7-20	26	N	N	N	G	G	G	N	N	B	G	N

Table 4.9: Results of assigning ecological Reserve categories to PES Na_2SO_4 data for the Upper Olifants river reaches using different BVs derived in chapter 3 and corresponding invertebrate assessment categories

PES site weir numbers	River reach	Na_2SO_4 mg/L	SSD_{AF} Acute + AF	SSD_{ACR} Acute + ACR	SSD_{LRAA} Acute + LRA	SSD_J Jooste & Rossouw (2002)	SSD_P Palmer et al. (2004c)	Bioassement category assigned for invertebrates (DWAF, 2000b): Category according to A/B/C/D/E/F classification	Bioassement category assigned for invertebrates (DWAF, 2000b): Category according to N/G/F/P classification	TDS category (DWAF, 2000b)
B1H 018 Q01	Olifants 1-8	36	N	N	N	G	N	C	G/F	F
B1H 005 Q01	Olifants 9-13	162	F	N	N	P	N	E	P	F
B1H 012 Q01	Klein Olifants 1-4	53	G	N	N	P	N	D	F	P
B1H 015 Q01	Klein Olifants 5-12	59	G	N	N	P	N	C	G/F	F
B2H 003 Q01	Wilge 1-6	No value returned	N	N	N	N	N	C	G/F	N
B2H 015 Q01	Wilge 7-20	27	N	N	N	G	N	B	G	N

4.5 Discussion

According to the results provided by the G-power calculation (table 4.7) present ecological state data for weirs B1H018Q01 (Olifants 1-8), B1H005Q01 (Olifants 9-13) and B2H015Q01 (Wilge 7-20) yielded a low confidence and all data provided by these weirs should be interpreted with caution.

According to Jooste and Rossouw's boundary values (2002) NaCl did not prove to be a concern as these values yielded only categories of good and natural (table 4.8). High concentrations of NaCl are largely associated with agricultural activities. High concentrations of NaCl might be expected in the middle and lower catchments of the Olifants due to irrigation activities and return flows in certain reaches of these catchments (DWAF, 2000b). This would require further investigation using DWAF ionic data and Jooste's salt model (SaltBA23.exe) to calculate present NaCl concentrations. The summaries in tables 4.8 for assigning categories for NaCl using the boundary values derived in chapter 3 show that there was no discrimination in resultant Reserve categories using acute data with an AF (SSD_{AF}), an ACR (SSD_{ACR}) and acute data with LRA (SSD_{LRAA}), as all the categories yielded the river reaches in a natural condition.

According to the boundary values proposed by Jooste and Rossouw (2002), the poor categories yielded by PES data for Na_2SO_4 for the Olifants segments 9-13 and the Klein Olifants segments 1-4 and 5-12, reflect the impact of the mining activities in these reaches of the river which are associated with high sulphate concentrations in the river. This is confirmed by the TDS assessment which yielded categories of fair or poor for these reaches (table 4.9). DWAF (2002) also found TDS and sulphate concentrations increased where streams passed through mining areas. For Na_2SO_4, the only guideline values derived from a particular SSD that showed some reflection of high sulphate concentrations in the regions dominated by coal mining activities, was SSD_{AF} using acute data with an AF and the BVs proposed by Jooste and Rossouw (2002) (table 4.9). SSD_{ACR} and SSD_{LRAA} yielded categories of natural for Na_2SO_4 for all segments of the Upper Olifants catchment, despite known problems of elevated Na_2SO_4, suggesting that the BVs generated by these SSDs were underprotective.

The results yielded by SSD_{AF}, SSD_{ACR} and SSD_{LRAA} categorised the river segments into ecological Reserve categories representing lower degrees of modification from the natural than what the biomonitoring and TDS results were indicating. This suggests that the values provided by SSD_{AF}, SSD_{ACR} and SSD_{LRAA} were possibly under-

protective. This advocates that the current use of acute data with either, AF, ACRs or LRA may be under-protective and not providing sufficient environmental realism. In view of the fact that acute data are far more prevalent than chronic data, possible suggestions for using acute data in the future would be to:

- Use an AF higher than 10 (some suggestions include 100, 1000 (CCME, 1991)).
- Exclude sub-chronic data in deriving ACRs as this results in the derivation of a very low ACR which results in high, under-protective BVs.
- Report acute data with complete concentration-response data over time so as to increase the sample size of data that can be extrapolated using LRA.

For both salts, tables 4.8 and 4.9 show that all reaches of the Upper Olifants were categorised as natural using SSD_P, i.e. the Australian and New Zealand method followed by Palmer *et al*. (2004a). In contrast, river reaches ranged from good, fair or poor according to both the invertebrate bioassessment and the TDS assessment. This suggests that either the BVs derived using SSD_P were under-protective, or that salts other than NaCl and Na_2SO_4 were contributing to instream toxicity. Disparities in the results may have been introduced due to the fact that data records identical to those used in the original Olifants assessment were not used (the report did not allow this), however, according to the Reserve determination, water quality concerns in these river reaches include high concentrations of TDS and sulphate, low pH, and at times high concentrations of iron, manganese and aluminium as a result of mining activities (DWAF, 1998). To fully evaluate the role of different types of data in the derivation of WQGs, the concentrations of all the toxicologically important major salts would need to be assessed, with particular focus on other sulphate salts, $MgSO_4$ and $CaSO_4$.

Table 4.8 shows that NaCl-based SSD_{LRAC}, SSD_{LOEC} to SSD_{NOEC} most accurately reflect the results of the invertebrate bioassessment, suggesting the use of sub-chronic data with LRA might prove to be most useful of guideline derivation. This despite the fact that catchment conditions suggest sulphate-based salts are more likely threatening invertebrate communities due to open-cast mining in the area (Jooste and Rossouw, 2002). A lack of Na_2SO_4 data to generate SSDs using LRA and NOEC and LOEC data (SSD_{LRAC}, SSD_{LOEC} and SSD_{NOEC}) did not allow for a comprehensive comparison in the suitability of different types of NaCl and Na_2SO_4 data to derive BVs. Further research would require the generation of either sub-chronic, NOEC or LOEC Na_2SO_4 data to facilitate a comprehensive comparison. As discussed in chapter 3, sub-chronic data are

easier to generate than either LOEC or NOEC data because they have shorter experimental lengths and usually have mortality as a comparatively, easily-measurable endpoint. In lieu of the fact that chronic data involve lengthy, costly and complex experiments, further research would provide the most valuable contribution to guideline derivation by providing sub-chronic data, reported as time-response data to facilitate LRA.

4.6 Conclusions

This chapter has provided provisional insight into the role of different types of data in deriving WQGs and in particular has focused on the interpretation and application of toxicological data in guideline derivation. Acute data can play a valuable role in establishing guidelines because acute data are more numerous than chronic data and represent a wider range of taxa than cub-chronic and chronic data. However, the use of AF and ACR applied to acute data needs careful consideration as the magnitude of these greatly affect the final BV derived and can have serious implications for water resource managers. For example, if either is too small, they can result in under-protective guidelines.

Two aspects which have not been addressed here due to time constraints are that of seasonality and site-specificity. Firstly, rivers are variable systems and rivers in South Africa are particularly vulnerable to extreme high and low flows associate with extreme flooding and drought events (Davies and Day, 1998a; Rowntree, 2000), which can affect instream salt concentrations. Provisional methods have been developed to link flows to concentrations (Malan and Day, 2002) and ideally should be considered in further guideline development. Secondly, as discussed in chapter 1, there are various natural drivers for salinisation. These can vary from catchment to catchment depending on regional climatic events and underlying geology. Hence, the need for water resource managers to address salinisation at a catchment level or site-specific level is highlighted here. Aquatic invertebrates are known to have evolved complex osmo-regulatory mechanisms (Chapman, 1998) and might have naturally high salinity tolerances in areas that have naturally saline environments. Hence, there is a need to address site-specificity when deriving WQGs.

This chapter has also highlighted the valuable contribution the use of SSDs and reconstituting inorganic salts has made to refining WQGs for salinity. By incorporating exposure durations and using both methods conjunctively in the future, they can be

used to complete comprehensive risk assessments for salinity, i.e. assessing the risk of organisms being exposed to selected inorganic salts based on the time organisms are exposed to certain concentrations of that salt. To provide comprehensive risk assessments, attention also needs to be given to the instream toxicity of a mixture of salts together with other toxicants, at specific sites giving rise to site-specific risk assessments.

Criticisms of the SSD approach do exist (Forbes and Forbes, 1993) and address the lack on any structure in the model accounting for interspecies relationships and ecosystem functioning, the problem of bioavailability of the toxicant, as well as methodological criticisms on the statistical components, i.e. distribution assumptions, the percentile estimated, the method of assessing uncertainty of percentiles and the fraction of species affected (Forbes and Forbes, 1993; Hopkin, 1993; Smith and Cairns, 1993). Despite these criticisms, the use of SSDs is more often preferred over traditional, non-statistical approaches such as the AF approach for deriving protective concentrations (Knoben *et al*., 1998, Kooijman, 1987, Newman *et al*., 2000, Okkerman *et al*., 1991, Posthuma *et al*., 2001, Schudoma, 1994) and the value of using SSDs in risk assessment studies has been recognised (Aldenberg and Jaworska, 2000; Grist *et al*., 2002; Wheeler *et al*., 2002).

CHAPTER 5: Concluding summary

The objectives of this study were met as follows:

1. **Expand the acute salinity toxicity database in South Africa by conducting acute toxicity tests using indigenous freshwater macroinvertebrates, particularly organisms not previously used in toxicity tests.**

The acute toxicity tests conducted in chapter 2 provided a valuable contribution towards the salinity tolerances of macroinvertebrates in South Africa. After data screening, 15 new datasets were generated for NaCl and 16 for Na_2SO_4. A total of 9 species were used that had not previously been used in toxicity tests and hence, provide valuable benchmarks for future testing. Results from the tests combined with other acute data for other indigenous species show that for both NaCl and Na_2SO_4, responses of indigenous freshwater macroinvertebrates are within the response ranges of species represented in the international USEPA toxicity database.

Furthermore, as a result of the toxicity tests conducted, experimental methods were developed for testing organisms that had not previously been used in acute toxicity tests, particularly the predacious Coenagrionidae. Most of the test organisms in this study proved to be suitable test organisms with the exception of Pleiidae and the Simulid larvae. Repeated toxicity tests using the same organisms, toxicants, experimental designs, diluents, and organisms collected from the same locations, could be conducted to contribute towards further assessing the variability in responses introduced from field-collected organisms. Issues of variability in particular could include seasonality, site-specificity and testing different life stages. Further tests would also assess the validity of using the lentic experimental system to develop a protocol for organisms collected from lentic systems.

2. **Evaluate the role of acute toxicity data in the development of water quality guidelines for salinity.**

Different guideline values for two inorganic salts, derived with different types of data manipulations of acute toxicity data, were examined. Chronic data and extrapolation techniques were also included and the different values obtained relative to other guideline values has been discussed. Acute tolerance data generated from this study

were applied with both AFs and ACRs for both salts to produce SSDs so as to generate protective concentration (PC) values that could equate to South Africa's ecological health categories. Other types of data used to derive SSDs included LOEC, NOEC and extrapolated data. It was found that both national and international toxicity response data for NaCl and Na_2SO_4 were dominated by acute datasets. The strength of the SSD lies in the input data representing various levels of organism functioning naturally occurring in the environment and covering a wide range of taxonomic groups. A large proportion of the South African acute response data were represented by mayfly tolerance data (22% of the data for both salts) and future tests would make a valuable contribution towards the SSD approach by selecting test organisms that represent an even broader range of taxonomic groups.

Combined national and international acute data for both salts were representative of a wide range of taxonomic groups, including annelids, crustaceans, fish, insects, flatworms, molluscs, nematodes and plants. However, acute data provide the least environmental realism, as organisms are typically exposed to long-term exposures of low concentrations of toxicants in the field. Hence, SSDs were generated using data providing more environmental realism, such as LOEC and NOEC data. However, due to the complexity of generating such data (long term experimental lengths, no easily measured endpoints, etc), such data were limited and sample sizes small, resulting in a decreased confidence in the data.

The value of focusing future toxicity testing on generating sub-chronic data was highlighted. Sub-chronic data could be generated with less 'effort' and complexity than chronic experiments but provide more environmental realism than acute data. The need for reporting comprehensive concentration-response data over time (i.e. the duration of the experiment) was also highlighted due to the contribution such data could make towards acute to chronic extrapolations such as LRA.

Guideline values generated by the various types of data were compared by applying them to a case study in the upper Olifants River catchment. Using DWAF ionic data and the SaltBA23.exe model (Jooste, 2004, pers comm.) ionic data were reconstituted and expected concentrations for NaCl and Na_2SO_4 for various reaches of the catchment were calculated. Based on these calculations, ecological health classes were assigned for each river reach and compared to the proposed boundary values generated by the various SSDs as well as the findings of the invertebrate and TDS assessments (carried out previously as part of a comprehensive Reserve determination

for the Olifants River catchment). Acute toxicity data applied with AFs and ACRs were found to be underprotective when compared to biomonitoring data. Sufficient Na_2SO_4 data were not available to provide any concluding recommendations. For NaCl, BVs derived using extrapolated sub-chronic data, LOEC and NOEC data, closely reflected the results of the invertebrate bioassessment. However, to fully evaluate the role of different types of data in deriving WQGs for salinity, all toxicologically important salts would need to be incorporated in a case study such as the upper Olifants, and not only NaCl and Na_2SO_4.

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APPENDICES

Appendix 1: Notes from Personal Communication with curators of the National Freshwater Invertebrate collection, Albany Museum, Grahamstown.

Caenid sp.1 identification – H. James

I have identified your Caenidae. They belong to a group I have called Caenid sp. 1. I have written descriptions of 10 different species from different parts of the country. Whenever I find one that doesn't fit what I've described already, I describe it, so the list is growing gradually. The trouble with caenids is that many have been described as adults only, without the nymph. If the nymphs are described and they turn out to belong to those adults, we just get lists of synonyms. Very few are described as nymphs.

Appendix 2: Mean 96 hour Electrical Conductivity (EC, mS/m), pH, Temperature (Temp, ºC) and Dissolved Oxygen (DO, mg/L) values, Experiments 1-11.

Experiment 1: Sodium chloride												
96 Hour Means	Experimental vessel/Concentration (mg/L):											
	0A	0B	0C	100	1000	3000	8000	4000	5000	6000	7000	12000
EC	51.9	50.1	54.4	72.9	241.2	489.0	800.0	802.2	1129.8	1272.6	1487.4	2134.0
Std Dev	2.6	10.4	2.2	5.0	7.0	237.3	19.6	169.9	64.9	38.4	33.9	46.2
pH	7.6	7.7	7.7	7.5	7.7	7.6	7.6	7.7	7.4	7.6	7.7	7.6
Std Dev	0.1	0.0	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1
Temp	17.9	17.6	16.8	17.9	17.5	18.1	16.7	16.5	17.6	16.4	17.2	16.9
Std Dev	0.6	0.8	0.9	0.7	1.0	0.6	0.9	1.0	0.8	1.0	0.9	0.9

Experiment 1: Sodium sulphate											
96 Hour Means	Experimental vessel/Concentration (mg/L):										
	0A	0B	100	500	1000	2000	3000	5000	6000	8000	10000
EC	55.1	53.9	69.7	130.3	190.8	326.4	448.2	674.8	799.8	1022.8	1219.4
Std Dev	1.5	2.1	2.1	2.9	3.1	7.8	8.9	34.8	12.2	23.1	20.5
pH	7.4	7.4	7.6	7.5	7.6	7.7	7.9	7.8	7.9	8.0	7.9
Std Dev	0.3	0.3	0.1	0.3	0.2	0.3	0.0	0.3	0.3	0.1	0.2
Temp	17.8	17.3	18.1	17.6	17.8	17.3	18.0	17.4	17.8	18.0	17.9
Std Dev	0.9	0.8	0.7	0.8	0.9	0.8	0.6	0.8	0.7	0.7	0.8

Experiment 2: Sodium chloride								
96 Hour Means	Experimental vessel/Concentration (mg/L):							
	0	100	1000	2000	5000	10000	20000	40000
EC	52.9	70.1	219.6	411.2	926.0	1708.6	3206.0	5646.0
Std Dev	2.8	3.4	5.8	4.8	16.3	27.2	114.4	193.2
pH	7.1	6.7	6.6	7.0	6.9	7.0	6.8	7.2
Std Dev	0.1	0.1	0.1	0.1	0.1	0.3	0.1	0.0
Temp	19.3	18.9	18.8	19.1	19.3	19.5	19.1	19.3
Std Dev	0.3	0.4	0.4	0.1	0.5	0.6	0.2	0.3

Experiment 3: Sodium chloride											
96 Hour Means	Experimental vessel/Concentration (mg/L):										
	0A	0B	0C	500	1500	3000	5000	6000	7000	8000	10000
EC	55.5	56.8	54.6	162.5	327.0	551.8	776.3	1037.0	1147.8	1308.5	1582.3
Std Dev	3.8	3.4	2.2	5.9	7.0	63.7	302.9	107.2	189.3	163.7	247.6
pH	7.1	6.9	6.6	7.2	7.0	6.5	7.2	6.9	7.1	7.1	6.7
Std Dev	0.1	0.1	0.2	0.1	0.1	0.0	0.1	0.1	0.2	0.1	0.2
Temp	18.7	18.3	18.2	18.5	18.6	18.2	18.7	18.5	18.5	18.7	18.3
Std Dev	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.1

Experiment 3: Sodium sulphate														
96 Hour Means	Experimental vessel/Concentration (mg/L):													
	0A	0B	0C	100	500	1000	2000	3000	4000	5000	8000	15000	25000	40000
EC	56.0	55.6	55.7	71.6	131.8	197.0	315.5	429.0	540.8	620.8	949.3	1633.3	1911.0	2890.0
Std Dev	2.9	1.9	2.4	2.6	6.8	4.0	6.6	32.8	28.2	43.8	106.0	116.0	715.1	1230.4
pH	7.2	7.2	7.3	7.3	7.3	7.4	7.4	7.5	7.5	7.5	7.6	7.5	7.6	7.6
Std Dev	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Temp	18.4	18.2	18.1	18.7	18.7	17.8	18.3	17.6	18.6	17.7	17.9	17.3	17.5	17.2
Std Dev	0.1	0.1	0.0	0.3	0.3	0.2	0.1	0.4	0.2	0.2	0.2	0.3	0.4	0.4

Experiment 4: Sodium chloride										
96 Hour	Experimental vessel/Concentration (mg/L):									
Means	0	5	10	15	20	23	27	30	35	45
EC	55.2	852.5	1589.8	1755.4	2534.5	3223.0	3261.3	3720.5	4785.5	4587.8
Std Dev	2.7	266.2	155.7	1172.5	1135.2	642.5	1285.0	1237.8	1221.3	2627.3
pH	7.2	6.7	7.1	7.0	6.9	7.0	6.9	6.9	6.8	6.9
Std Dev	0.2	0.5	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2
Temp	18.0	17.3	17.7	18.1	17.3	18.0	17.5	18.1	17.4	17.9
Std Dev	0.5	0.9	0.7	0.5	0.9	0.6	0.8	0.5	0.9	0.7

Experiment 4: Sodium sulphate										
96 Hour	Experimental vessel/Concentration (mg/L):									
Means	0	5	15	20	24	28	32	35	40	45
EC	55.0	613.3	860.3	612.8	1557.9	1282.0	1993.5	1800.3	1879.5	3151.0
Std Dev	6.3	55.7	521.3	802.4	1140.8	757.1	1472.3	920.6	878.2	1723.6
pH	6.9	7.2	7.4	7.4	7.4	7.6	7.6	7.8	7.7	7.7
Std Dev	0.1	0.3	0.2	0.2	0.3	0.2	0.0	0.3	0.2	0.1
Temp	17.6	16.9	17.1	17.1	18.2	17.3	17.0	17.6	17.9	17.0
Std Dev	0.7	0.9	0.9	0.9	1.0	0.8	0.9	0.7	0.7	0.9

Experiment 5: Sodium chloride								
96 Hour	Experimental vessel/Concentration (mg/L):							
Means	0	100	500	1000	5000	10000	20000	40000
EC	43.0	64.4	127.8	237.4	801.2	1587.8	3088.0	6020.0
Std Dev	1.8	3.1	3.5	2.2	7.5	10.4	100.3	42.4
pH	7.6	7.7	7.5	7.6	7.5	7.4	7.2	6.8
Std Dev	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1
Temp	18.9	18.7	18.9	18.9	18.7	18.7	18.7	18.4
Std Dev	0.7	0.6	0.7	0.6	0.6	0.7	0.8	0.7
DO	6.9	7.0	6.5	6.8	6.4	6.2	7.1	5.7
Std Dev	0.7	0.3	0.7	0.8	0.6	0.9	0.8	0.4

Experiment 5: Sodium sulphate								
96 Hour	Experimental vessel/Concentration (mg/L):							
Means	0	100	500	1000	5000	10000	20000	40000
EC	35.8	57.1	110.1	172.6	654.8	1211.4	2166.0	3745.0
Std Dev	1.5	1.0	5.7	5.3	14.5	16.6	39.7	35.4
pH	7.6	7.7	7.7	7.7	7.9	7.9	8.0	8.5
Std Dev	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.4
Temp	18.5	18.6	18.7	18.3	18.7	18.4	18.4	18.9
Std Dev	0.6	0.6	0.6	0.6	0.5	0.5	0.6	0.5
DO	6.4	6.2	6.5	6.8	7.0	6.3	5.9	7.0
Std Dev	1.0	0.9	0.9	0.5	0.4	0.7	0.4	0.6

Experiment 6: Sodium chloride										
96 Hour	Experimental vessel/Concentration (mg/L):									
Means	0	500	1000	2000	4000	6000	8000	10 000	15 000	30 000
EC	46.8	135.3	248.0	443.5	812.0	1164.0	1514.8	1831.8	2710.0	4943.3
Std Dev	0.6	2.7	8.8	5.6	12.4	11.9	9.9	12.1	24.5	56.9
pH	8.0	7.9	7.9	7.8	7.1	7.7	7.7	7.7	7.4	7.4
Std Dev	0.2	0.2	0.2	0.2	0.1	0.3	0.2	0.3	0.1	0.2
Temp	19.3	19.3	19.0	19.4	18.6	19.3	19.2	19.4	18.7	19.0
Std Dev	0.4	0.2	0.3	0.2	0.5	0.1	0.2	0.2	0.3	0.1
DO	8.9	9.2	8.9	8.6	7.8	8.7	8.8	7.9	8.3	7.8
Std Dev	1.0	1.9	1.6	1.3	1.2	1.8	1.1	1.4	2.0	0.8

Experiment 6: Sodium sulphate										
96 Hour	Experimental vessel/Concentration (mg/L):									
Means	0	500	1000	2000	4000	6000	8000	10 000	15 000	30 000
EC	45.9	114.8	176.6	340.3	459.8	837.3	1050.0	1281.0	1764.0	3200.0
Std Dev	0.6	1.2	4.6	6.6	275.9	13.6	15.0	15.6	15.3	38.3
pH	7.9	7.7	7.8	7.9	7.9	7.9	8.0	8.0	8.1	8.2
Std Dev	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Temp	19.2	18.2	18.6	19.0	18.8	18.3	19.0	18.4	19.2	18.5
Std Dev	0.3	0.3	0.3	0.2	0.2	0.3	0.2	0.4	0.2	0.3
DO	8.7	9.6	9.8	9.6	9.6	9.9	8.6	9.8	8.9	9.7
Std Dev	1.0	3.3	1.5	1.3	1.3	3.0	1.2	3.3	1.4	1.7

Experiment 7: Sodium chloride										
96 Hour	Experimental vessel/Concentration (mg/L):									
Means	0	100	1000	2000	5000	6000	7000	8000	10 000	15 000	40 000
EC	45.9	48.1	215.4	369.2	1949.8	989.3	1265.0	1830.6	2214.6	2828.6	5306.4
Std Dev	4.9	5.1	12.6	14.6	2839.9	28.6	45.3	60.2	59.4	71.5	153.0
pH	7.7	7.7	7.4	7.6	7.6	7.5	7.4	7.5	7.5	7.4	7.3
Std Dev	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1
Temp	18.2	18.2	17.4	18.0	18.2	18.1	17.6	17.8	18.1	18.1	18.3
Std Dev	0.6	0.7	0.6	0.6	0.6	0.7	0.6	0.7	0.6	0.7	0.7
DO	7.9	8.3	8.6	8.9	7.8	8.4	7.9	7.2	7.7	7.9	7.4
Std Dev	1.5	0.6	0.7	0.9	1.2	1.0	0.7	0.3	0.8	0.6	1.4

Experiment 7: Sodium sulphate										
96 Hour	Experimental vessel/Concentration (mg/L):									
Means	0	100	1000	2000	5000	6000	7000	8000	10 000	15 000	40 000
EC	46.7	61.5	169.2	282.7	596.8	696.3	792.1	884.5	1054.4	1474.8	3218.8
Std Dev	4.9	3.7	4.2	4.5	12.8	21.0	22.5	26.0	35.9	52.5	119.3
pH	7.6	7.5	7.6	7.7	7.8	7.7	7.7	7.8	7.9	7.9	8.2
Std Dev	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.3
Temp	17.8	17.4	17.9	18.1	17.8	17.1	17.5	17.3	18.1	17.7	17.4
Std Dev	0.6	0.7	0.7	0.6	0.6	0.8	0.6	0.6	0.7	0.6	0.6
DO	9.0	9.0	8.5	7.8	9.2	8.8	8.5	8.3	8.2	7.9	6.9
Std Dev	0.7	1.2	1.2	1.5	1.1	1.8	1.3	1.2	1.2	0.8	0.9

Experiment 8: Sodium chloride									
96 Hour	Experimental vessel/Concentration (mg/L):								
Means	0	50	100	300	400	500	600	800	12000
EC	48.8	1144.4	2116.2	2920.5	3285.3	3768.8	4176.3	4753.5	5835.5
Std Dev	1.8	23.1	323.3	42.7	47.1	53.0	53.0	39.7	67.4
pH	7.8	7.6	7.3	7.4	7.5	7.1	7.3	7.3	7.3
Std Dev	0.1	0.0	0.1	0.1	0.1	0.2	0.1	0.1	0.0
Temp	18.4	18.6	17.9	18.5	18.6	17.7	18.2	18.5	18.5
Std Dev	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.3
DO	7.3	8.1	7.6	6.5	7.7	7.0	8.0	5.9	6.8
Std Dev	0.9	1.1	0.8	0.7	0.7	1.0	1.1	0.9	0.9

Experiment 8: Sodium sulphate									
96 Hour	Experimental vessel/Concentration (mg/L):								
Means	0	50	100	300	400	500	600	800	12000
EC	52.1	1053.5	1866.5	2170.5	2425.8	2732.8	2918.0	3243.8	3595.5
Std Dev	1.8	21.6	22.2	25.6	32.2	20.8	37.4	36.3	29.1
pH	7.6	7.7	7.9	7.9	7.9	8.1	8.0	8.1	8.0
Std Dev	0.1	0.1	0.3	0.1	0.1	0.2	0.2	0.2	0.1
Temp	18.5	17.4	17.6	18.2	17.6	18.3	17.4	17.9	17.7
Std Dev	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2
DO	5.3	8.3	6.7	7.2	6.5	6.0	6.3	6.2	5.9
Std Dev	0.6	1.0	1.4	1.6	0.6	1.2	0.9	1.4	1.2

Experiment 9: Sodium chloride										
96 Hour	Experimental vessel/Concentration (mg/L):									
Means	0	500	1000	2000	2800	3200	3500	4000	5000	8000
EC	49.3	132.6	217.4	375.3	499.8	560.8	603.9	669.6	811.4	1670.3
Std Dev	1.1	4.5	6.3	7.5	14.0	16.3	15.2	19.2	22.2	39.4
pH	7.6	7.6	7.5	7.5	7.5	7.5	7.5	7.4	7.4	7.4
Std Dev	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1
Temp	17.8	17.0	16.6	17.6	17.6	17.6	17.6	16.5	17.3	16.8
Std Dev	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
DO	10.4	10.3	10.4	10.0	9.9	9.6	10.4	9.9	10.7	10.2
Std Dev	0.6	0.8	1.0	1.0	0.8	1.0	1.3	1.3	1.4	1.6

Experiment 9: Sodium sulphate											
96 Hour	Experimental vessel/Concentration (mg/L):										
Means	0	500	2000	3000	4000	5000	6000	7000	8000	10 000	12 000
EC	49.7	108.7	280.2	390.8	488.6	585.2	683.7	769.5	843.4	1045.7	1192.7
Std Dev	2.6	1.9	5.9	9.9	5.3	9.1	6.3	9.9	12.2	16.6	20.5
pH	7.6	7.5	7.6	7.6	7.7	7.7	7.8	7.7	7.7	7.8	7.8
Std Dev	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Temp	17.5	16.0	16.5	16.0	16.9	16.3	16.7	16.1	16.0	16.5	16.0
Std Dev	0.1	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.3	0.2	0.2
DO	10.2	9.8	10.5	10.8	9.7	11.3	10.4	10.3	11.0	10.9	10.2
Std Dev	1.5	0.8	1.8	1.7	0.4	1.1	2.1	1.2	1.6	2.3	1.6

Experiment 10: Sodium sulphate									
96 Hour	Experimental vessel/Concentration (mg/L):								
Means	0	50	100	300	400	500	600	800	12000
EC	52.4	113.6	174.6	401.4	514.1	607.3	694.6	891.5	1229.5
Std Dev	1.8	3.7	5.1	11.8	15.7	13.6	15.2	25.4	35.1
pH	7.8	7.9	7.9	7.7	7.9	7.8	8.0	7.9	7.8
Std Dev	0.2	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Temp	17.2	16.8	17.0	17.4	17.3	17.6	17.1	17.3	17.5
Std Dev	0.5	0.5	0.4	0.5	0.5	0.4	0.5	0.5	0.5
DO	8.9	8.7	8.7	8.9	8.8	8.7	8.4	8.4	8.5
Std Dev	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.4	0.6

Experiment 11: Sodium chloride (channels)								
96 Hour	Experimental vessel/Concentration (mg/L):							
Means	0	50	100	400	700	10000	20000	40000
EC	50.9	137.6	222.9	690.7	1156.6	1583.9	2953.6	5382.4
Std Dev	6.0	7.8	10.6	28.3	45.0	55.5	76.4	167.3
pH	7.7	7.7	7.7	7.6	7.5	7.5	7.4	7.2
Std Dev	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Temp	18.5	18.4	18.7	18.6	17.9	18.3	18.9	18.0
Std Dev	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.2
DO	8.1	8.0	8.4	8.0	8.1	8.3	7.7	8.1
Std Dev	0.8	0.7	0.9	0.9	0.7	0.7	0.8	0.8

Experiment 11: Sodium chloride (tanks)								
96 Hour	Experimental vessel/Concentration (mg/L):							
Means	0	50	100	400	700	10000	20000	40000
EC	60.3	139.5	222.5	710.2	1161.6	1588.1	2455.5	5448.4
Std Dev	4.6	5.7	7.8	19.0	27.1	35.5	1209.4	103.3
pH	7.85	7.81	7.73	7.62	7.58	7.53	7.43	7.36
Std Dev	0.12	0.24	0.14	0.11	0.12	0.13	0.15	0.13
Temp	18.2	17.8	18.4	18.1	18.4	18.4	18.4	18.0
Std Dev	0.1	0.2	0.4	0.4	0.3	0.4	0.3	0.4
DO	8.0	8.1	7.9	8.2	8.2	7.7	7.7	7.7
Std Dev	0.8	0.6	0.7	0.9	0.6	0.6	0.7	0.5

Appendix 3: Reported ranges of RQS water quality data

Sodium chloride

		Exp 1	Exp 5	Exp 6	Exp 7	Exp 8	Exp 9	Exp 11	Exp 11
Major inorganic determinants	**Units**							Tanks	Channels
pH	pH units	6.8-7.5	5.0-7.5	6.5-7.5	7.1-7.6	7.2-7.7	7.1-7.6	7.1-7.5	7.3-7.7
Kjeldahl nitrogen as N	mg/L	-	0.32-1.12	0.48-0.82	0.46-1.38	0.42-0.58	0.42-0.56	<0.30-0.51	<0.30-0.39
Ammonium as N	mg/L	<0.04-0.64	<0.03	<0.03-0.07	<0.03-0.13	<0.03-0.04	<0.03-0.04	<0.03	<0.03
Nitrate + nitrite as N	mg/L	<0.04-0.99	<0.11-.0.22	<0.11	<0.11-0.37	<0.11	<0.11	<0.11	<0.11
Flouride as F	mg/L	0.1-0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2
Alkalinity as calcium carbonate	mg/L	22-74	29-93	28-86	11-65	35-99	30-58	35-61	38-64
Sodium as Na	mg/L	53-3185	58-16485	64-13967	16-3442	76-13313	70-3553	90-15573	80-16436
Magnesium as Mg	mg/L	1-12	<1-15	7-14	6-238	11-227	1-56	11-13	12-14
Silicon as Si	mg/L	2.7-2.9	2.4-3.4	2.5-3.1	1.9-2.2	1.8-2.1	1.8-2.1	1.8-2.1	2.0-2.3
Total phosphorous as P	mg/L	-	<0.03-0.948	0.030-0.170	<0.030	<0.030-0.383	<0.030-0.090	<0.030-0.036	<0.030-0.031
Ortho phosphate as P	mg/L	0.015-0.042	<0.023-0.082	<0.023-0.050	<0.023-0.056	<0.023-0.064	0.023	<0.023-0.029	<0.023-0.025
Sulphate as SO4	mg/L	54-217	12-249	17-277	23-349	48-756	45-120	37-51	34-265
Chloride as Cl	mg/L	101-7986	108-24927	118-20477	36-11807	131-21251	124-5359	159-24303	144-24679
Potassium as K	mg/L	3.1-6.7	3.0-19.2	3.0-5.4	2.1-78.5	3.2-80.1	0.4-12.1	3.4-6.2	3.5-4.8
Calcium as Ca	mg/L	22-52	17-63	18-53	10-343	23-352	3-33	20-26	22-107
Electrical Conductivity	mS/m	52.6-1840	54.6-5610	56.9-4500	0.2-5690	60.5-6120	59.6-1550	72-5330	67.1-5470
Trace metals (all reported as dissolved)									
B	mg/L	<0.012-0.043	<0.026-0.053	<0.026-0.129	<0.026-0.100	0.040-0.098	0.057-0.130	<0.026-0.060	<0.026-0.063
Al	mg/L	<0.052-0.099	<0.018	<0.018	<0.018-0.059	<0.479	<0.018-0.020	<0.479-0.152	<0.018-0.120
V	mg/L	<0.007	<0.022	<0.022	<0.022	<0.022	<0.022-0.022	<0.022-0.027	<0.022-0.027
Cr	mg/L	<0.005	<0.016	<0.016	<0.016	<0.016	<0.016	<0.016	<0.016
Mn	mg/L	<0.001	<0.012	<0.012	<0.012	<0.012	<0.012-0.234	<0.012	<0.012
Fe	mg/L	<0.005-0.162	<0.027-0.123	<0.027	<0.027-2.174	<0.027	<0.027-8.340	<0.027-0.066	<0.027-6.78
Ni	mg/L	<0.015	<0.032	<0.032	<0.032-0.032	<0.032	<0.032	<0.032	<0.032-0.054
Cu	mg/L	<0.011	<0.044	<0.044-0.082	<0.044-0.245	<0.044	<0.044-0.045	<0.044	<0.044-0.090
Zn	mg/L	<0.007-0.156	<0.014-0.315	<0.014-0.021	<0.014	<0.014	<0.014	<0.014	<0.014-5.060
Sr	mg/L	0.108-0.123	0.084-0.120	0.107-0.129	0.123-0.141	0.127-0.146	0.148-0.167	0.135-0.159	0.140-0.184
Mo	mg/L	<0.012	<0.037	<0.037	<0.037	<0.037	<0.037	<0.037	<0.037
Cd	mg/L	<0.008	<0.009	<0.009	<0.009	<0.009	<0.009	<0.009	<0.009
Ba	mg/L	<0.013	<0.014	<0.014-0.019	<0.014	<0.014	<0.014	<0.014	<0.014
Pb	mg/L	<0.071	<0.126	<0.126	<0.126	<0.126	<0.126	<0.126	<0.126-0.220

Sodium sulphate

		Exp 1	Exp 5	Exp 6	Exp 7	Exp 8	Exp 9	Exp 10
Major inorganic determinants	**Units**							
pH	pH units	7.0-7.6	7.1-8.0	7.2-7.9	7.1-8.1	7.7-8.1	7.5-7.8	6.7-7.7
Kjeldahl nitrogen as N	mg/L	-	0.52-1.43	0.59-1.31	0.44-0.87	0.46-0.84	0.44-0.65	-
Ammonium as N	mg/L	<0.04	<0.03	<0.03	<0.03-0.08	<0.03-0.04	<0.030-0.19	<0.03-0.17
Nitrate + nitrite as N	mg/L	<0.04-0.07	<0.11	<0.11	<0.11	<0.11	<0.11-0.14	<0.11-0.39
Flouride as F	mg/L	0.1-0.2	<0.2-0.3	<0.2-0.3	<0.2-0.3	<0.2-0.3	<0.2-0.2	<0.2-0.5
Alkalinity as calcium carbonate	mg/L	22-131	29-139	24-226	34-117	31-289	30-96	42-109
Sodium as Na	mg/L	53-2712	58-6953	62-9923	61-5120	80-12429	74-3892	16-4887
Magnesium as Mg	mg/L	7-12	7-115	7-15	10-123	11-245	12-16	4-13
Silicon as Si	mg/L	2.7-2.9	2.9-4.1	2.6-3.4	2.1-2.8	2.2-2.9	2.1-2.6	1.5-3.9
Total phosphorous as P	mg/L	-	0.030-29.188	<0.030-18.902	0.105-36.337	0.498-30.211	0.036-9.514	-
Ortho phosphate as P	mg/L	<0.011-3.424	<0.023-8.493	<0.023-3.897	0.055-4.081	0.036-5.113	0.027-0.496	<0.023-0.053
Sulphate as SO4	mg/L	58-5995	45-13744	41-19024	14-10224	53-19191	51-7941	18-8575
Chloride as Cl	mg/L	87-111	29-326	113-300	29-402	130-1237	101-203	<5-144
Potassium as K	mg/L	3.2-5.1	3.1-21	2.9-9.5	3.2-40.5	3.7-79.4	3.3-8.0	<0.3-4.2
Calcium as Ca	mg/L	22-51	11-84	Aug-27	21-171	21-342	14-28	<1-29
Electrical Conductivity	mS/m	52.7-1219.40	55.1-3560	54.1-2980	0.2-3600	0.2-3950	60.6-1470	0.5-1440
Trace metals (all reported as dissolved)								
B	mg/L	0.022-6.460	<0.026-20.650	<0.026-9.34	0.093-20.80	0.081-22.730	0.089-8.030	<0.026-0.088
Al	mg/L	<0.083	<0.018	<0.018	<0.018-0.091	<0.018-0.072	<0.018	<0.018
V	mg/L	<0.007	<0.022	<0.022	<0.022	<0.022	<0.022-0.025	<0.022-0.023
Cr	mg/L	<0.005	<0.016	<0.016	<0.016	<0.016	<0.016	<0.016
Mn	mg/L	<0.001	<0.012	<0.012	<0.012	<0.012	<0.012	<0.012-0.055
Fe	mg/L	<0.005-0.170	<0.027-0.218	<0.027-0.138	<0.027-1.253	<0.027-0.444	<0.0270.161	<0.027-0.512
Ni	mg/L	<0.015-0.052	<0.032-0.278	<0.032-0.102	<0.032-0.294	0.032-0.0.340	<0.032-0.092	<0.032
Cu	mg/L	<0.011	<0.044	<0.044	<0.044	<0.044	<0.044	<0.044
Zn	mg/L	<0.007-0.011	<0.014	<0.014-0.290	<0.014	<0.014	<0.014	<0.014-0.302
Sr	mg/L	0.116-0.248	0.11	0.108-0.137	0.137-0.160	0.134-0.151	0.149-0.166	0.164-0.217
Mo	mg/L	<0.012	<0.037	<0.037	<0.037	<0.037	<0.037	<0.037
Cd	mg/L	<0.008	<0.009	<0.009	<0.009	<0.009	<0.009	<0.009-0.021
Ba	mg/L	<0.013-0.019	<0.014	<0.014-0.021	<0.014	<0.014	<0.014	<0.014
Pb	mg/L	<0.017	<0.126	<0.126	<0.126	<0.126	<0.126	<0.126

Appendix 4: Comprehensive list of control mortalities

Experiment No.	Salt	Organism	No. Responding	No. exposed	% mortality
1	NaCl	*Baetis harrisoni*	12	25	48.0
		Demoreptus natalensis	6	8	75.0
		Euthraulus elegans	6	85	7.1
		Oligoneuropsis lawrencei	7	90	7.8
	Na_2SO_4	*Afronurus barnardi*	2	50	4.0
		Burnupia stenochorias	2	21	9.5
		Euthraulus elegans	0	58	0.0
		Tricorythus discolor	1	49	2.0
		Oligoneuropsis lawrencei	7	90	7.8
2	NaCl	Coenagrionidae	1	26	3.8
3	NaCl	*Cloeon virgilae*	8	140	5.7
		Plea pullula	1	31	3.2
	Na_2SO_4	*Cloeon virgilae*	0	104.0	0.0
		Coenagrionidae	0	12	0.0
		Plea pullula	1	30	3.3
4	NaCl	Coenagrionidae	0	35	0.0
	Na_2SO_4	Coenagrionidae	0	39	0.0
5	NaCl	*Leptocerid* sp.	1	48	2.1
		Plea pullula	2	28	7.1
	Na_2SO_4	*Leptocerid* sp.	0	30	0.0
		Plea pullula	2	28	7.1
6	NaCl	*Leptocerid sp.*	0	46	0.0
	Na_2SO_4	*Leptocerid sp.*	0	36	0.0
7	NaCl	*Plea pullula*	22	39	56.4
	Na_2SO_4	*Plea pullula*	17	44	38.6
8	NaCl	Coenagrionidae	0	39	0.0
	Na_2SO_4	Coenagrionidae	0	38	0.0
9		*Baetis harrisoni*	1	25	4.0
		Burnupia stenochorias	1	26	3.8
	NaCl	*Demoreptus natalensis*	2	17	11.8
	Na_2SO_4	*Baetis harrisoni*	0	23	0.0
		Burnupia stenochorias	0	33	0.0
		Euthraulus elegans	1	38	2.6
10	Na_2SO_4	*Demoreptus natalensis*	9	15	60.0
		Oligoneuropsis lawrencei	2	20	10.0
11	NaCl	*Suragine* sp.	0	12	0.0
		Caenid sp.1	0	9	0.0
		Simulidae	12	15	80.0

Appendix 5: Probit results

Experiment 1, NaCl

Euthraulus elegans

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
100	31	2	0.065	0.065	0.002
1000	28	2	0.071	0.071	0.077
3000	29	3	0.103	0.103	0.246
4000	27	4	0.148	0.148	0.310
5000	33	10	0.303	0.303	0.365
6000	25	1	0.040	0.040	0.412
7000	27	8	0.296	0.296	0.453
8000	28	21	0.750	0.750	0.488
12000	33	33	1.000	1.000	0.596

Chi - Square for Heterogeneity (calculated) = 138.049

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 3.922083

Sigma = 0.646194

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-1.069513	5.14877	(-13.246355, 11.107329)
Slope	1.547523	1.377569	(-1.710428, 4.805474)

Theoretical Spontaneous Response Rate = 0.0000

Experiment 1, NaCl

Oligoneuropsis lawrencei

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	29	1	0.035	0.000	0.107
100	29	2	0.069	-0.042	0.000
1000	32	5	0.156	0.056	0.000
3000	30	6	0.200	0.105	0.067
4000	34	12	0.353	0.276	0.273
5000	24	13	0.542	0.487	0.536
6000	32	24	0.750	0.720	0.744
7000	34	31	0.912	0.901	0.872
8000	35	33	0.943	0.936	0.940
12000	36	36	1.000	1.000	0.998

Chi - Square for Heterogeneity (calculated) = 1.667

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Mu = 3.686518

Sigma = 0.139663

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-21.3958	4.168507	(-29.566072, -13.225526)
Slope	7.16009	1.112775	(4.979051, 9.341130)
Spontaneous Response Rate	0.106502	0.037755	(0.032501, 0.180502)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	2299.426	1515.465	2889.003
5	2862.766	2068.994	3430.32
10	3217.551	2440.09	3763.104
15	3481.536	2725.646	4008.469
50	4858.673	4286.102	5314.81
85	6780.543	6205.702	7653.526
90	7336.852	6664.084	8480.23
95	8246.113	7363.985	9928.729
99	10266.348	8800.505	13467.878

Experiment 1, NaCl

Demoreptus natalensis

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
100	7	2	0.286	0.286	0.216
1000	8	4	0.500	0.500	0.513
3000	5	4	0.800	0.800	0.664
4000	10	6	0.600	0.600	0.701
5000	6	0	0.000	0.000	0.728
6000	10	9	0.900	0.900	0.749
7000	6	6	1.000	1.000	0.766
12000	7	7	1.000	1.000	0.821

Chi - Square for Heterogeneity (calculated) = 21.717

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 12.592

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 2.959537

Sigma = 1.220539

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	2.575222	1.980779	(-2.271745, 7.422190)
Slope	0.81931	0.568888	(-0.572758, 2.211378)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	1.319		
5	8.951		
10	24.848		
15	49.496		
50	911.038		
85	16768.859		
90	33402.570		
95	92726.039		
99	629328.250		

Experiment 1, NaCl

Baetis harrisoni

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 1, Na_2SO_4

Euthraulus elegans

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls
100	26	1	0.039	0.039
500	30	0	0.000	0.000
1000	27	0	0.000	0.000
2000	27	2	0.074	0.074
3000	31	4	0.129	0.129
5000	27	2	0.074	0.074
6000	26	3	0.115	0.115
8000	27	14	0.519	0.519
10000	28	23	0.821	0.821

Chi - Square for Heterogeneity (calculated) = 643.425

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 4.007097

Sigma = 0.522932

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-2.662746	12.585157	(-32.426643, 27.101152)
Slope	1.912294	3.398571	(-6.125325, 9.949913)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	617.447		
5	1402.573		
10	2172.234		
15	2918.298		
50	10164.752		
85	35404.887		
90	47564.902		
95	73666.086		
99	167337.672		

Experiment 1, Na_2SO_4

Afronurus barnardi

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	50	2	0.040	0.000	0.081
100	29	4	0.138	0.062	0.000
500	31	4	0.129	0.052	0.000
1000	26	1	0.039	-0.047	0.004
2000	27	4	0.148	0.073	0.054
3000	27	5	0.185	0.113	0.156
5000	30	14	0.467	0.420	0.396
6000	23	14	0.609	0.574	0.502
8000	25	16	0.640	0.608	0.666
10000	29	23	0.793	0.775	0.775

Chi - Square for Heterogeneity (calculated) = 4.015

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 3.776639

Sigma = 0.295720

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-7.770988	2.675356	(-13.014685, -2.527290)
Slope	3.381575	0.70729	(1.995286, 4.767864)
Spontaneous Response Rate	0.08123	0.023629	(0.034916, 0.127544)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	1226.606	385.629	2017.349
5	1950.782	839.289	2827.867
10	2498.297	1266.430	3396.770
15	2952.26	1667.558	3853.753
50	5979.144	4873.850	7192.651
85	12109.416	9484.877	20161.617
90	14309.812	10780.586	26499.158
95	18326.063	12969.099	39924.691
99	29145.594	18203.174	86781.086

Experiment 1, Na_2SO_4

Tricorythus discolor

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	48	1	0.021	0.000	0.073
100	31	3	0.097	0.025	0.000
500	26	2	0.077	0.004	0.000
1000	25	3	0.120	0.050	0.000
2000	22	3	0.136	0.068	0.000
3000	24	2	0.083	0.011	0.001
5000	27	1	0.037	-0.039	0.035
6000	25	7	0.280	0.223	0.098
8000	20	5	0.250	0.191	0.321
10000	25	16	0.640	0.612	0.570

Chi - Square for Heterogeneity (calculated) = 7.305

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 3.973226

Sigma = 0.151046

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-21.304781	8.099029	(-37.178879, -5.430685)
Slope	6.620509	2.066207	(2.570742, 10.670276)
Spontaneous Response Rate	0.07331	0.021466	(0.031236, 0.115384)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	4186.476	1355.925	5576.458
5	5306.046	2472.479	6521.991
10	6020.685	3389.556	7124.245
15	6556.655	4175.122	7595.792
50	9402.131	8233.999	12187.459
85	13482.495	10967.249	28953.998
90	14682.727	11613.353	35909.484
95	16660.254	12617.370	49495.668
99	21115.629	14692.531	90647.930

Experiment 1, Na_2SO_4

Burnupia stenochorias

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 2, NaCl

Coenagrionidae

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 3, NaCl

Cloeon virgilae

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 3, NaCl

Plea pullula

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 3, Na_2SO_4

Cloeon virgilae

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 3, Na_2SO_4

Plea pullula

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 3, Na_2SO_4

Coenagrionidae

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
5000	12	0	0.000	0.000	0.001
8000	11	1	0.091	0.091	0.013
15000	12	0	0.000	0.000	0.113
25000	12	3	0.250	0.250	0.351
40000	7	6	0.857	0.857	0.647

Chi - Square for Heterogeneity (calculated) = 8.619

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 7.815

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 4.500833

Sigma = 0.268711

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-11.749692	8.909859	(-40.100861, 16.601477)
Slope	3.721465	2.043372	(-2.780544, 10.2234750

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	7511.546		
5	11450.628		
10	14336.799		
15	16685.533		
50	31683.457		
85	60162.379		
90	70018.523		
95	87666.93		
99	133639.719		

Experiment 4, NaCl

Coenagrionidae

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
5000	37	0	0.000	0.000	0.000
10000	39	0	0.000	0.000	0.000
15000	44	6	0.136	0.136	0.051
20000	39	7	0.180	0.180	0.370
23000	40	24	0.600	0.600	0.618
27000	40	33	0.825	0.825	0.847
30000	39	39	1.000	1.000	0.934
35000	36	36	1.000	1.000	0.986
45000	40	40	1.000	1.000	1.000

Chi - Square for Heterogeneity (calculated) = 16.182

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 4.332916

Sigma = 0.096007

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-40.131298	7.402067	(-57.637188, -22.625410)
Slope	10.415917	1.697861	(6.400477, 14.431357)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	12869.903	8843.521	15331.294
5	14962.166	11243.484	17178.297
10	16213.385	12759.734	18279.963
15	17116.488	13882.829	19082.691
50	21523.666	19376.605	23419.668
85	27065.613	24750.461	31406.309
90	28573.197	25928.896	34049.980
95	30962.645	27682.721	38514.836
99	35996.250	31113.408	48816.453

Experiment 4, Na_2SO_4

Coenagrionidae

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
5000	40	0	0.000	0.000	0.000
15000	39	0	0.000	0.000	0.005
20000	39	3	0.077	0.077	0.106
24000	38	17	0.447	0.447	0.342
28000	40	21	0.525	0.525	0.619
32000	39	35	0.897	0.897	0.820
35000	35	31	0.886	0.886	0.908
40000	40	38	0.950	0.950	0.974
45000	40	40	1.000	1.000	0.994

Chi - Square for Heterogeneity (calculated) = 6.886

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Mu = 4.418697

Sigma = 0.094413

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-41.801739	4.654032	(-50.923641, -32.679836)
Slope	10.59175	1.047483	(8.538684, 12.644816)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	15814.820	13713.366	17458.744
5	18339.910	16436.662	19816.746
10	19847.123	18089.428	21217.770
15	20933.773	19288.029	22230.273
50	26223.904	25025.164	27364.990
85	32850.895	31287.947	34957.047
90	34649.516	32830.668	37216.820
95	37497.086	35202.781	40899.742
99	43484.098	40013.297	48953.426

Experiment 5, NaCl

Leptocerid sp.

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 5, NaCl

Plea pullula

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	28	2	0.071	0.000	0.072
100	22	3	0.136	0.069	0.000
500	29	1	0.035	-0.041	0.001
1000	22	1	0.046	-0.029	0.010
5000	27	16	0.593	0.561	0.315
10000	30	10	0.333	0.281	0.623
20000	29	28	0.966	0.963	0.866
40000	23	23	1.000	1.000	0.971

Chi - Square for Heterogeneity (calculated) = 24.428

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 11.070

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 0.881733

Sigma = 0.379119

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	2.67426	1.297939	(-0.662743, 6.011262)
Slope	2.637691	1.216791	(0.490679, 5.766061)
Spontaneous Response Rate	0.072299	0.059385	(-0.080379, 0.224976)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	1.000		
5	1.812		
10	2.488		
15	3.082		
50	7.616		
85	18.821		
90	23.314		
95	32.015		
99	58.034		

Experiment 5, Na_2SO_4

Leptocerid sp.

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
100	50	2	0.040	0.040	0.001
500	53	5	0.094	0.094	0.029
1000	48	1	0.021	0.021	0.079
5000	47	6	0.128	0.128	0.385
10000	44	14	0.318	0.318	0.576
20000	46	45	0.978	0.978	0.750
40000	47	47	1.000	1.000	0.877

Chi - Square for Heterogeneity (calculated) = 114.442

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 11.070

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 3.881074

Sigma = 0.622669

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-1.232961	2.952713	(-8.824386, 6.358463)
Slope	1.605989	0.755948	(-0.337552, 3.549530)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	270.743		
5	719.192		
10	1210.767		
15	1720.835		
50	7604.553		
85	33605.332		
90	47762.461		
95	80408.602		
99	213594.156		

Experiment 5, Na_2SO_4

Plea pullula

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	28	2	0.071	0.000	0.096
100	35	4	0.114	0.020	0.000
500	28	3	0.107	0.013	0.000
1000	29	3	0.103	0.008	0.000
5000	28	4	0.143	0.052	0.129
10000	25	18	0.720	0.690	0.548
20000	33	29	0.879	0.866	0.915
40000	31	31	1.000	1.000	0.996

Chi - Square for Heterogeneity (calculated) = 3.764

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 11.070

Mu = 0.971042

Sigma = 0.240221

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	0.957713	0.849684	(0.707667, 2.623094)
Slope	4.162832	0.786252	(2.621778, 5.703886)
Spontaneous Response Rate	0.095854	0.026726	(0.043471, 0.148237)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	2.584	1.030	4.021
5	3.766	1.853	5.355
10	4.604	2.526	6.259
15	5.273	3.107	6.968
50	9.355	7.117	11.487
85	16.596	13.421	22.998
90	19.007	15.135	27.926
95	23.237	17.903	37.614
99	33.874	24.110	66.900

Experiment 6, NaCl

Leptocerid sp.

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
500	18	0	0.000	0.000	0.000
1000	28	0	0.000	0.000	0.000
2000	39	1	0.026	0.026	0.000
4000	39	1	0.026	0.026	0.040
6000	32	5	0.156	0.156	0.219
8000	34	13	0.382	0.382	0.468
10000	30	21	0.700	0.700	0.677
15000	42	41	0.976	0.976	0.925
30000	43	43	1.000	1.000	0.999

Chi - Square for Heterogeneity (calculated) = 86.526

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 3.917597

Sigma = 0.179781

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-16.790981	9.279749	(-38.737587, 5.155626)
Slope	5.562334	2.368054	(-0.038114, 11.162781)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	3157.717		
5	4186.738		
10	4866.201		
15	5386.073		
50	8271.743		
85	12703.442		
90	14060.606		
95	16342.494		
99	21668.092		

Experiment 6, Na_2SO_4

Leptocerid sp.

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
500	44	0	0.000	0.000	0.000
1000	41	0	0.000	0.000	0.000
2000	43	0	0.000	0.000	0.000
4000	41	0	0.000	0.000	0.000
6000	43	2	0.047	0.047	0.015
8000	44	5	0.114	0.114	0.117
10000	40	9	0.225	0.225	0.334
15000	42	37	0.881	0.881	0.829
30000	48	48	1.000	1.000	1.000

Chi - Square for Heterogeneity (calculated) = 5.822

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 14.067

Mu = 1.054816

Sigma = 0.127621

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-3.265191	1.010606	(-5.245978, -1.284404)
Slope	7.835675	0.988447	(5.898319, 9.773031)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	5.727	4.638	6.548
5	6.997	6.008	7.744
10	7.785	6.878	8.492
15	8.367	7.520	9.056
50	11.345	10.555	12.343
85	15.384	13.886	17.949
90	16.534	14.758	19.691
95	18.397	16.131	22.615
99	22.475	19.019	29.385

Experiment 7, NaCl

Plea pullula

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 7, Na_2SO_4

Plea pullula

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 8, NaCl

Coenagrionidae

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
7000	37	0	0.000	0.000	0.000
15000	38	3	0.079	0.079	0.085
20000	36	20	0.556	0.556	0.495
23000	41	28	0.683	0.683	0.741
27000	40	37	0.925	0.925	0.920
30000	37	36	0.973	0.973	0.971
35000	33	33	1.000	1.000	0.996
45000	37	37	1.000	1.000	1.000

Chi - Square for Heterogeneity (calculated) = 1.433

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 12.592

Mu = 1.302252

Sigma = 0.091991

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-9.156373	1.694387	(-12.477372, -5.835374)
Slope	10.870687	1.264088	(8.393074, 13.348299)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	12.253	10.258	13.745
5	14.156	12.335	15.499
10	15.288	13.599	16.536
15	16.103	14.518	17.283
50	20.056	18.945	21.046
85	24.980	23.725	26.705
90	26.312	24.865	28.430
95	28.416	26.598	31.262
99	32.828	30.067	37.499

Experiment 8, Na_2SO_4

Coenagrionidae

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
10000	39	0	0.000	0.000	0.000
20000	39	5	0.128	0.128	0.135
24000	40	24	0.600	0.600	0.543
28000	39	31	0.795	0.795	0.871
32000	37	37	1.000	1.000	0.978
35000	37	37	1.000	1.000	0.996
40000	39	39	1.000	1.000	1.000
45000	41	41	1.000	1.000	1.000

Chi - Square for Heterogeneity (calculated) = 3.567

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 12.592

Mu = 1.373074

Sigma = 0.065410

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-15.991827	2.631568	(-21.149700, -10.833954)
Slope	15.288193	1.890694	(11.582433, 18.993954)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	16.631	14.576	18.073
5	18.428	16.653	19.674
10	19.465	17.866	20.599
15	20.197	18.727	21.257
50	23.609	22.633	24.505
85	27.597	26.476	29.188
90	28.635	27.361	30.549
95	30.246	28.686	32.731
99	33.515	31.268	37.344

Experiment 9, NaCl

Baetis harrisoni

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	25	1	0.040	0.000	0.059
500	20	4	0.200	0.150	0.073
1000	8	2	0.250	0.203	0.265
2000	19	11	0.579	0.553	0.578
2800	22	15	0.682	0.662	0.725
3200	22	19	0.864	0.855	0.775
4000	23	16	0.696	0.677	0.846
5000	23	23	1.000	1.000	0.901
8000	21	21	1.000	1.000	0.968

Chi - Square for Heterogeneity (calculated) = 10.032

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 12.592

Mu = 0.229175

Sigma = 0.365165

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.372406	0.27813	(3.827271, 4.917542)
Slope	2.738488	0.536596	(1.686761, 3.790215)
Spontaneous Response Rate	0.059029	0.045308	(-0.029775, 0.147832)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	0.240	0.048	0.499
5	0.425	0.120	0.761
10	0.577	0.196	0.955
15	0.709	0.272	1.114
50	1.695	1.062	2.205
85	4.052	3.214	5.627
90	4.979	3.885	7.551
95	6.758	5.014	11.979
99	11.986	7.816	29.469

Experiment 9, NaCl

Demoreptus natalensis

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 9, NaCl

Burnupia stenochorias

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	26	1	0.039	0.000	0.019
1000	19	0	0.000	-0.019	0.000
2000	19	0	0.000	-0.019	0.020
2800	23	8	0.348	0.335	0.154
3200	23	4	0.174	0.158	0.271
3500	22	6	0.273	0.259	0.370
4000	22	13	0.591	0.583	0.531
5000	22	17	0.773	0.768	0.778
8000	18	18	1.000	1.000	0.987

Chi - Square for Heterogeneity (calculated) = 9.276

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 12.592

Mu = 0.590998

Sigma = 0.141070

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	0.810615	0.740797	(-0.641348, 2.262578)
Slope	7.08866	1.282225	(4.575498, 9.601821)
Spontaneous Response Rate	0.018826	0.019256	(-0.018916, 0.056568)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	1.832	1.215	2.243
5	2.285	1.703	2.657
10	2.572	2.034	2.914
15	2.785	2.288	3.107
50	3.899	3.572	4.299
85	5.460	4.829	6.869
90	5.913	5.142	7.740
95	6.653	5.634	9.254
99	8.302	6.665	12.978

Experiment 9, NaCl

Demoreptus natalensis

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 9, Na_2SO_4

Burnupia stenochorias

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
500	19	3	0.158	0.158	0.061
2000	19	3	0.158	0.158	0.357
3000	22	9	0.409	0.409	0.493
4000	17	13	0.765	0.765	0.590
5000	26	13	0.500	0.500	0.662
6000	25	19	0.760	0.760	0.717
8000	25	23	0.920	0.920	0.794

Chi - Square for Heterogeneity (calculated) = 14.934

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 11.070

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 0.486648

Sigma = 0.508067

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	4.042158	0.427378	(2.943368, 5.140947)
Slope	1.968243	0.660307	(0.270594, 3.665891)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	0.202	0.000	0.838
5	0.448	0.000	1.316
10	0.685	0.000	1.689
15	0.912	0.000	2.013
50	3.067	0.430	6.272
85	10.309	5.403	6472.522
90	13.734	6.557	50121.344
95	21.007	8.533	1065014.875
99	46.619	13.549	339295904.000

Experiment 9, Na_2SO_4

Euthraulus elegans

Note : No convergence.

This usually means that a probit model is not appropriate for your concentration response data

Experiment 9, Na_2SO_4

Baetis harrisoni

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
500	23	0	0.000	0.000	0.000
2000	23	0	0.000	0.000	0.001
3000	21	1	0.048	0.048	0.028
4000	25	5	0.200	0.200	0.175
5000	22	5	0.227	0.227	0.429
6000	20	17	0.850	0.850	0.669
8000	20	18	0.900	0.900	0.921

Chi - Square for Heterogeneity (calculated) = 7.128

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 11.070

Mu = 0.722010

Sigma = 0.128554

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-0.616406	0.91227	(-2.404455, 1.171643)
Slope	7.778845	1.227583	(5.274782, 10.282907)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	2.648	1.914	3.154
5	3.240	2.560	3.698
10	3.608	2.981	4.036
15	3.880	3.298	4.290
50	5.272	4.836	5.801
85	7.165	6.397	8.695
90	7.705	6.787	9.636
95	8.580	7.396	11.241
99	10.497	8.660	15.056

Experiment 10, Na_2SO_4

Demoreptus natalensis

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	15	9	0.600	0.000	0.383
50	16	4	0.250	-0.215	0.001
100	9	2	0.222	-0.260	0.029
300	20	17	0.850	0.757	0.551
400	18	13	0.722	0.550	0.745
600	15	14	0.933	0.892	0.920
800	21	21	1.000	1.000	0.974
1200	19	19	1.000	1.000	0.996

Chi - Square for Heterogeneity (calculated) = 6.464

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 11.070

Mu = 2.447185

Sigma = 0.235234

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-5.403197	4.082962	(-13.405802, 2.599408)
Slope	4.251088	1.549717	(1.213642, 7.288533)
Spontaneous Response Rate	0.38288	0.080162	(0.225762, 0.539997)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	79.426	1.039	168.163
5	114.881	3.751	210.443
10	139.865	7.421	237.715
15	159.731	11.743	258.471
50	280.017	78.960	381.008
85	490.887	352.274	846.477
90	560.610	418.994	1224.524
95	682.529	504.240	2273.683
99	987.209	658.922	7860.909

Experiment 10, Na_2SO_4

Oligoneuropsis lawrencei

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
Control	20	2	0.100	0.000	0.078
50	17	1	0.059	-0.021	0.002
100	14	1	0.071	-0.008	0.018
300	18	6	0.333	0.277	0.179
400	17	5	0.294	0.234	0.272
600	20	8	0.400	0.349	0.432
800	17	10	0.588	0.553	0.555
1200	18	14	0.778	0.759	0.717

Chi - Square for Heterogeneity (calculated) = 1.680

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 11.070

Mu = 2.847762

Sigma = 0.403871

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-2.051163	2.205005	(-6.372973, 2.270648)
Slope	2.476037	0.786516	(0.934466, 4.017608)
Spontaneous Response Rate	0.078437	0.041787	(-0.003466, 0.160341)

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	80.954	2.334	189.930
5	152.558	12.326	284.920
10	213.875	29.742	355.937
15	268.652	53.611	415.835
50	704.306	482.738	1075.256
85	1846.434	1166.801	10357.967
90	2319.333	1360.802	18702.686
95	3251.543	1697.656	45191.172
99	6127.559	2543.872	238890.625

Experiment 11, NaCl

Suragina sp.

Concen-tration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
500	8	0	0.000	0.000	0.000
1000	11	0	0.000	0.000	0.000
4000	11	0	0.000	0.000	0.000
7000	11	0	0.000	0.000	0.008
10000	10	0	0.000	0.000	0.062
20000	7	6	0.857	0.857	0.571
40000	10	9	0.900	0.900	0.971

Chi - Square for Heterogeneity (calculated) = 4.877

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 11.070

Mu = 1.269660

Sigma = 0.175347

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-2.240826	1.822587	(-5.813096, 1.331444)
Slope	5.702963	1.451923	(2.857195, 8.548732)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	7.274	2.871	10.430
5	9.577	4.831	12.895
10	11.090	6.316	14.578
15	12.244	7.520	15.937
50	18.606	14.074	25.952
85	28.274	21.315	52.225
90	31.217	23.117	62.680
95	36.148	25.925	82.602
99	47.596	31.794	140.140

Experiment 11, NaCl

Caenid sp.1

Concentration (mg/L)	Number exposed	Number Respond	Observed proportion responding	Proportion responding adjusted for controls	Predicted proportion responding
500	9	1	0.111	0.111	0.016
1000	7	0	0.000	0.000	0.082
4000	8	2	0.250	0.250	0.548
10000	9	9	1.000	1.000	0.868
20000	8	8	1.000	1.000	0.970

Chi - Square for Heterogeneity (calculated) = 10.339

Chi - Square for Heterogeneity (tabular value at 0.05 level) = 7.815

Warning: The tabular chi-square value exceeds the calculated chi-square value for heterogeneity.

This is evidence that the probit model may not be appropriate for these data.

The results reported for this data set may not be valid, and should be interpreted with appropriate caution.

Note: Slope not significantly different from zero, LC/EC fiducial limits cannot be computed.

Mu = 0.554419

Sigma = 0.398196

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	3.607673	0.868138	(0.845258, 6.370090)
Slope	2.511324	1.128636	(-1.079996, 6.102643)

Theoretical Spontaneous Response Rate = 0.0000

Estimated LC/EC Values and Confidence Limits

LC/EC	Exposure Concentration	95% Confidence Limits	
		Lower	Upper
1	0.425		
5	0.793		
10	1.107		
15	1.386		
50	3.584		
85	9.271		
90	11.608		
95	16.196		
99	30.251		

Appendix 6: Trimmed-Spearman Kärber results

Experiment 1	NaCl	
	Euthraulus elegans	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	30	0
100	31	2
1000	28	2
3000	29	3
4000	27	4
5000	33	10
6000	25	1
7000	27	8
8000	28	21
10000	33	33

SPEARMAN-KARBER TRIM: 6.45%

SPEARMAN-KARBER ESTIMATES: LC50: 6674.82

95% LOWER CONFIDENCE: 5177.52

95% UPPER CONFIDENCE: 8605.13

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 1	NaCl	
	Demoreptus natalensis	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	8	6
100	5	4
1000	6	2
3000	4	4
4000	8	4
5000	6	6
6000	9	8
7000	6	6
12000	8	8

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 4.37

95% LOWER CONFIDENCE: 3.49

95% UPPER CONFIDENCE: 5.47

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 1	NaCl	
	Baetis harrisoni	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	25	12
100	9	4
1000	6	3
3000	11	10
4000	11	10
5000	16	15
6000	13	12
7000	16	16
8000	18	18
12000	9	9

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 1.95

95% LOWER CONFIDENCE: 1.35

95% UPPER CONFIDENCE: 2.80

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 1	**Na_2SO_4**	
	Euthraulus elegans	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	58	0
100	26	1
500	30	0
1000	27	0
2000	27	2
3000	31	4
5000	27	2
6000	26	3
8000	27	14
10000	28	23

SPEARMAN-KARBER TRIM: 17.86%

SPEARMAN-KARBER ESTIMATES: LC50: 7907.97

95% LOWER CONFIDENCE: 7271.97

95% UPPER CONFIDENCE: 8599.60

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 1	**Na_2SO_4**	
	Burnupia stenochorias	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	21	2
100	9	0
500	8	0
1000	12	2
2000	10	1
3000	9	1
5000	6	1
6000	8	8
8000	8	8
12000	10	10

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 4580.17

95% LOWER CONFIDENCE: 3786.45

95% UPPER CONFIDENCE: 5540.28

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

Experiment 2	**NaCl**	
	Coenagrionidae	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	26	1
100	28	1
1000	28	0
2000	28	0
5000	28	0
10000	27	0
20000	27	6
40000	16	16

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 24.41

95% LOWER CONFIDENCE: 21.88

95% UPPER CONFIDENCE: 27.22

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 3	NaCl	
	Cloeon virgilae	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	42	0
500	51	1
1500	44	2
3000	49	1
5000	35	16
6000	47	41
7000	56	54
8000	61	61
10000	60	60

SPEARMAN-KARBER TRIM: 1.96%

SPEARMAN-KARBER ESTIMATES: LC50: 4.68

95% LOWER CONFIDENCE: 4.28

95% UPPER CONFIDENCE: 5.12

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 3	Na_2SO_4	
	Cloeon virgilae	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	42	0
100	31	1
500	35	0
1000	39	1
2000	32	6
3000	36	8
4000	35	24
5000	44	34
8000	50	50

SPEARMAN-KARBER TRIM: 1.52%

SPEARMAN-KARBER ESTIMATES: LC50: 3.37

95% LOWER CONFIDENCE: 2.99

95% UPPER CONFIDENCE: 3.79

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 3	Na_2SO_4	
	Coenagrionidae	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	12	0
5000	12	0
8000	11	1
15000	12	0
25000	12	3
40000	7	6

SPEARMAN-KARBER TRIM: 14.29%

SPEARMAN-KARBER ESTIMATES: LC50: 29926.71

95% LOWER CONFIDENCE: 24223.67

95% UPPER CONFIDENCE: 36972.42

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 4	NaCl	
	Coenagrionidae	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	35	0
5000	37	0
10000	39	0
15000	44	6
20000	39	7
23000	40	24
27000	40	33
30000	39	39
35000	36	36
45000	40	40

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 21397.26

95% LOWER CONFIDENCE: 20300.01

95% UPPER CONFIDENCE: 22553.81

Experiment 5	NaCl	
	Leptocerid sp.	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	48	1
100	49	0
500	32	2
1000	41	15
5000	51	5
10000	47	26
20000	47	47
40000	47	47

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 5.62

95% LOWER CONFIDENCE: 4.43

95% UPPER CONFIDENCE: 7.14

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 5	NaCl	
	Plea pullula	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	28	2
100	22	3
500	29	1
1000	22	1
5000	27	16
10000	30	10
20000	29	28
40000	23	23

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 6.74

95% LOWER CONFIDENCE: 5.22

95% UPPER CONFIDENCE: 8.71

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION

Experiment 5	Na_2SO_4	
	Leptocerid sp.	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	30	0
100	50	2
500	53	5
1000	48	1
5000	47	6
10000	44	14
20000	46	45
40000	47	47

SPEARMAN-KARBER TRIM: 4.00%

SPEARMAN-KARBER ESTIMATES: LC50: 9.72

95% LOWER CONFIDENCE: 7.91

95% UPPER CONFIDENCE: 11.95

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 6	NaCl	
	Leptocerid sp.	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	46	0
500	18	0
1000	28	0
2000	39	1
4000	39	1
6000	32	5
8000	34	13
10000	30	21
15000	42	41
30000	43	43

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 8.29

95% LOWER CONFIDENCE: 7.53

95% UPPER CONFIDENCE: 9.12

Experiment 7	NaCl	
	Plea pullula	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	39	22
100	48	32
1000	44	13
2000	40	15
4000	44	21
6000	40	27
8000	45	27
12000	45	30
15000	40	38
20000	44	43
40000	44	44

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 10.19

95% LOWER CONFIDENCE: 9.30

95% UPPER CONFIDENCE: 11.16

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 7	Na_2SO_4	
	Plea pullula	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	44	17
100	42	18
1000	50	24
2000	51	26
5000	44	24
6000	47	21
7000	45	22
8000	44	20
10000	39	21
15000	47	36
40000	41	41

SPEARMAN-KARBER TRIM: 6.88%

SPEARMAN-KARBER ESTIMATES: LC50: 10.00

95% LOWER CONFIDENCE: 7.63

95% UPPER CONFIDENCE: 13.10

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 9	NaCl	
	Demoreptus natalensis	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	17	2
500	15	3
1000	17	1
2000	14	3
2800	16	9
3500	15	4
4000	21	5
5000	21	18
8000	19	19

SPEARMAN-KARBER TRIM: .83%

SPEARMAN-KARBER ESTIMATES: LC50: 3.80

95% LOWER CONFIDENCE: 3.32

95% UPPER CONFIDENCE: 4.34

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 9	Na_2SO_4	
	Baetis harrisoni	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	23	0
500	19	3
2000	19	3
3000	22	9
4000	17	13
5000	26	13
6000	25	19
8000	25	23

SPEARMAN-KARBER TRIM: 15.79%

SPEARMAN-KARBER ESTIMATES: LC50: 3647.38

95% LOWER CONFIDENCE: 3091.39

95% UPPER CONFIDENCE: 4303.37

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.
ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 9	**Na_2SO_4**	
	Euthraulus elegans	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	38	1
500	35	1
2000	33	1
4000	30	1
6000	35	4
7000	35	7
8000	33	6
10000	34	21
12000	45	42

SPEARMAN-KARBER TRIM: 6.85%

SPEARMAN-KARBER ESTIMATES: LC50: 9264.77

95% LOWER CONFIDENCE: 8759.17

95% UPPER CONFIDENCE: 9799.56

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Experiment 11	**NaCl**	
	Caenid sp.1	
Concentration (mg/L)	**Number Exposed**	**Mortalities**
0	9	0
500	9	1
1000	7	0
4000	8	2
10000	9	9
20000	8	8

SPEARMAN-KARBER TRIM: 6.25%

SPEARMAN-KARBER ESTIMATES: LC50: 4.80

95% LOWER CONFIDENCE: 3.13

95% UPPER CONFIDENCE: 7.35

NOTE: MORTALITY PROPORTIONS WERE NOT MONOTONICALLY INCREASING.

ADJUSTMENTS WERE MADE PRIOR TO SPEARMAN-KARBER ESTIMATION.

Appendix 7: Summaries of cleaned and extracted data from the USEPA AQUIRE database

NaCl

Scientific name	Common name	Phylum	Sub-Phylum	Order	Geomean (mg/L)	No. of data points
Nais variabilis	Copepod	Arthropoda	Crustacea	Calanoida	5.84	1
Limnodrilus hoffmeisteri	Mayfly	Arthropoda	Hexapoda	Ephemeroptera	520.00	1
Erpobdella punctata	Striped bass	Chordata	Vertebrata	Perciformes	1206.08	9
Tubifex tubifex	Turbellarian, flatworm	Platyhel-minthes		Tricladida	1230.00	2
Epischura baikalensis	Rainbow trout	Chordata	Vertebrata	Salmoniformes	1282.57	19
Astacus astacus	European crayfish	Arthropoda	Crustacea	Decapoda	1402.63	1
Ceriodaphnia dubia	Water flea	Arthropoda	Crustacea	Diplostraca	1539.72	5
Streptocephalus rubricaudatus	Caddisfly	Arthropoda	Hexapoda	Trichoptera	1714.06	2
Cyclops vernalis	Ciliate Protozoa	Ciliophora		Hymeno-stomatida	1767.77	2
Asellus communis	Diatom	Kingdom: Plantae	Division: Bacillariophyta	Naviculales	2430.00	1
Streptocephalus proboscideus	Water flea	Arthropoda	Crustacean	Diplostraca	2454.01	3
Daphnia pulex	Mayfly	Arthropoda	Hexapoda	Ephemeroptera	2500.00	1
Daphnia magna	Shiner	Chordata	Vertebrata	Cypriniformes	2500.00	1
Lirceus fontinalis	Oligochaete	Annelida		Haplotaxida	2569.00	1
Leptodora kindtii	Ciliate	Ciliophora		Hymeno-stomatida	2922.15	2
Tricorythus sp.	Algae	Kingdom:Plantae		Zygnematales	2922.15	1
Chimarra sp.	Frog	Chordata	Vertebrata	Anura	3005.37	39
Stenonema rubrum	Fairy shrimp	Arthropoda	Crustacea	Anostraca	3070.00	1
Chironomus attenuatus	Water flea	Arthropoda	Crustacean	Diplostraca	3121.39	24
Hydroptila angusta	Pond snail	Mollusca		Basommatophora	3399.98	2
Baetis tricaudatus	Midge	Arthropoda	Hexapoda	Diptera	3437.09	8
Hydropsyche sp.	Flatly coiled gyraulus	Mollusca		Basomma-tophora	3440.93	2
Culex sp.	Rotifer	Rotifera		Ploima	3664.38	1
Argia sp.	Aquatic sowbug	Arthropoda	Crustacean	Isopoda	3688.17	2
Cricotopus trifasciatus	Water flea	Arthropoda	Crustacean	Diplostraca	3700.00	1

NaCl (cont.)

Scientific name	Common name	Phylum	Sub-Phylum	Order	Geomean (mg/L)	No. of data points
Geotrichum candidum	Cyclopoid	Arthropoda	Crustacea	Cyclopoida	4299.19	2
Morone saxatilis	Pouch snail	Mollusca		Basomma-tophora	5060.00	1
Oncorhynchus mykiss	Pond snail, pneumonate snail	Mollusca		Basomma-tophora	5713.56	13
Notropis sp.	Aquatic sowbug	Arthropoda	Crustacea	Isopoda	6486.52	4
Rana breviceps	Tubificid worm, Oligochaete	Annelida		Haplotaxida	6528.59	4
Pimephales promelas	Ramshorn snail	Mollusca		Basomma-tophora	6570.58	3
Lepomis macrochirus	Caddisfly	Arthropoda	Hexapoda	Trichoptera	6621.00	1
Carassius auratus	Mayfly	Arthropoda	Hexapoda	Ephemeroptera	6785.02	15
Danio rerio	Fairy shrimp	Arthropoda	Crustacea	Anostraca	6896.27	1
Micropterus salmoides	Planarian	Platyhelminthes		Tricladida	6966.00	2
Stizostedion lucioperca	Fathead minnow	Chordata	Vertebrata	Cypriniformes	6979.75	46
Gambusia holbrooki	Green algae	Kingdom: Plantae		Cladophorales	7305.38	1
Carassius carassius	Bluegill	Chordata	Vertebrata	Perciformes	7390.36	6
Hypophthalmichthys molitrix	Red leech	Annelida		Arhynchob-dellida	7500.00	3
Poecilia latipinna	Goldfish	Chordata	Vertebrata	Cypriniformes	8348.66	59
Gambusia affinis	Zebra danio	Chordata	Vertebrata	Cypriniformes	8928.65	4
Anguilla rostrata	Caddisfly	Arthropoda	Hexapoda	Trichoptera	9000.00	1
Cyprinus carpio	Tubificid worm	Annelida		Haplotaxida	9972.83	3
Poecilia reticulata	Largemouth bass	Chordata	Vertebrata	Perciformes	10000.00	1
Silurus glanis	Pikeperch	Chordata	Vertebrata	Perciformes	10000.00	4
Anguilla anguilla	Mosquito	Arthropoda	Hexapoda	Diptera	10348.91	2
Acipenser baerii	Eastern mosquitofish	Chordata	Vertebrata	Cyprinodonti-formes	11540.00	1
Cyprinidae	Green algae	Kingdom:Plantae		Chlorococcales	11688.60	7
Leuciscus cephalus	Crucian carp	Chordata	Vertebrata	Cypriniformes	13750.00	1
Leuciscus leuciscus	Silver carp	Chordata	Vertebrata	Cypriniformes	15566.24	5
Phoxinus phoxinus	Sailfin molly	Chordata	Vertebrata	Cyprinodonti-formes	17632.56	2
Rutilus rutilus	Western mosquitofish	Chordata	Vertebrata	Cyprinodonti-formes	17914.78	3
Eleginus navaga	American eel	Chordata	Vertebrata	Anguilliformes	19583.82	2

NaCl (cont.)

Scientific name	Common name	Phylum	Sub-Phylum	Order	Geomean (mg/L)	No. of data points
Gadus ogac	Nematode	Nemata		Rhabditida	21295.26	9
pungitius pungitius		Chordata	Vertebrata	Cypriniformes	21548.58	7
Anarhichas lupus	Guppy	Chordata	Vertebrata	Cyprinodonti-formes	22360.68	2
Heros severus	Wels, european catfish	Chordata	Vertebrata	Siluriformes	23400.00	1
Liopsetta glacialis	Damselfly	Arthropoda	Hexapoda	Odonata	25676.82	6
Salmo trutta	Common eel	Chordata	Vertebrata	Anguilliformes	26207.41	3
Salvelinus alpinus erythrinus	Fungi	Kingdom: Fungi	Division: Myxomycota	Saprolegniales	30000.00	1
Salvelinus namaycush	Fungus	Ascomycota	Ascomycotina	Saccharomy-cetales	37403.52	1
Thymallus thymallus	Grass carp, white amur	Chordata		Cypriniformes	47907.36	2
Oncorhynchus keta	Midge	Arthropoda	Hexapoda	Diptera	62210.00	1
Ctenopharyngodon idella	Siberian Sturgeon	Chordata	Vertebrata	Acipenseri-formes	101106.39	2
Tetrahymena thermophila	Minnow,carp family	Chordata	Vertebrata	Cypriniformes	101106.39	2
Paramecium tetraurelia	Chub	Chordata	Vertebrata	Cypriniformes	101106.39	1
Saprolegnia sp.	Dace	Chordata	Vertebrata	Cypriniformes	101106.39	1
Navicula seminulum	Minnow	Chordata	Vertebrata	Cypriniformes	101106.39	1
Pithophora oedogonia	Roach	Chordata	Vertebrata	Cypriniformes	101106.39	1
Spirogyra setiformis	Atlantic navaga	Chordata	Vertebrata	Gadiformes	101106.39	1
Chlorella emersonii	Greenland cod	Chordata	Vertebrata	Gadiformes	101106.39	4
Lymnaea sp.	10-spined stickleback	Chordata	Vertebrata	Gasterostei-formes	101106.39	1
Gyraulus circumstriatus	Atlantic wolf-fish	Chordata	Vertebrata	Perciformes	101106.39	1
Physa gyrina	Banded cichlid	Chordata	Vertebrata	Perciformes	101106.39	
Physa heterostropha	Arctic flounder	Chordata	Vertebrata	Pleuronecti-formes	101106.39	1
Helisoma campanulatum	Brown trout	Chordata	Vertebrata	Salmoniformes	101106.39	2
Caenorhabditis elegans	Char	Chordata	Vertebrata	Salmoniformes	101106.39	1
Polycelis nigra	Lake trout, siscowet	Chordata	Vertebrata	Salmoniformes	101106.39	1
Dugesia gonocephala	European Grayling	Chordata	Vertebrata	Salmoniformes	101106.39	1
Brachionus calyciflorus	Chum salmon	Chordata	Vertebrata	Salmoniformes	101106.39	1

Geomean = Geometric mean

Na_2SO_4

Scientific name	Common name	Phylum	sub-phylum	order	Geomean (mg/L)	Sample size (n)
Morone saxatilis	Striped bass	Chordata	Vertebrata	Perciformes	420.00	16
Oncorhynchus mykiss	Rainbow trout	Chordata	Vertebrata		704.00	1
Amphipoda	Scud order	Arthropoda	Crustacean	Amphipod	1195.96	4
Navicula seminulum	Diatom	Plantae (kingdom)			1900.00	1
Ceriodaphnia dubia	Water flea	Arthropoda	Crustacean	Diplostraca	3265.84	3
Daphnia magna	Water flea	Arthropoda	Crustacean	Diplostraca	3711.61	12
Lymnaea sp.	Pond snail	Mollusca		Basommatophora	4863.67	4
Gambusia affinis	Western mosquitofish	Chordata	Vertebrata	Cyprinodontiformes	5150.89	6
Polycelis nigra	Planarian	Platyhelminthes		Tricladida	6817.92	1
Lepomis macrochirus	Bluegill	Chordata	Vertebrata	Perciformes	9841.29	8
Culex sp.	Mosquito	Arthropoda	Hexapoda	Diptera	12352.75	2
Poecilia latipinna	Sailfin molly	Chordata	Vertebrata	Cyprinodontiformes	17904.18	2

Geomean = Geometric mean