Benchmarking a Decision Support System for Aquatic Toxicity Testing

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

The National Water Act mandates the protection of water resources in the country. This requirement is managed by the National Water Resource Strategy, which provides for a number of programmes addressing water physicochemistry, eutrophication, microbiological and toxicological risk, and ecosystem protection. The various programmes have been implemented to differing extent. Physicochemical monitoring is well established, biomonitoring programmes have been put into place, but implementation of toxicological monitoring has lagged. The National Toxicity Monitoring Programme has commenced monitoring toxicological risk to the resource, but formalized toxicological monitoring of effluent is still applied in an ad-hoc fashion. The Direct Estimation of Ecological Effect Potential was an approach formulated to undertake toxicological assessment of effluent to assess oxygen demand, acute risk (mortality), reproductive inhibition, mutagenicity, bioaccumulation and persistence potential in line with international best practice. Detailed methods for most these tests were published in 2004, but the suite of methods were never formally adopted. Further research in support of the initiation of formalized toxicity testing has been undertaken since. Ongoing decreases in South African water quality underscore the urgency of properly implementing all mandated water quality management systems.

More recently, a toxicological research project culminated in the production of a system which, based on user input, can output a recommended sampling regime, a test battery for each sample, a means of combining the test results into an ecologically meaningful classification system, and recommendations for compliance conditions. This tool is known as the Integrated Water Use Application Bioassay toolkit (IWUAB). Prior to potential implementation of the IWUAB toolkit in supporting toxicological monitoring of effluent, the current project was initiated to test the application of the toolkit in monitoring point and diffuse effluents from four economic sectors, and to introduce the toolkit to regulators and potential users.

Three broad approaches were taken to address the aims of the project. A pilot test of sample collection and assessment was undertaken to assess the performance of the IWUAB toolkit with point and diffuse effluents from four economic sectors. The IWUAB toolkit interface and approach was reviewed. Finally stakeholders were engaged to present the IWUAB toolkit, to offer input into the toolkit, and to be trained in the application of the toolkit.

AIMS

The aims of the project follow:

1. To demonstrate the application and implementation of the Integrated Water Use Authorisation Bioassay (IWUAB) Toolkit in a number of locations and industries.
2. To develop and build capacity on the use of the developed system and aquatic toxicity testing in general.
3. To collect toxicological data over one hydrological year and to prepare the reports that will enhance the work of the regulator in assessing compliance/non-compliance.
4. To highlight quality assurance practices associated with aquatic toxicity testing.

5. To set catchments-based limits in selected catchments through stakeholder engagement and dialogue.

6. To refine the developed decision support system (IWUAB Toolkit) for potential application by DWS and CMAs for water use authorisation.

METHODOLOGY

Stakeholders in the mining, municipal, industrial and agricultural sectors were approached to collaborate in allowing access to effluent points and to assist with sample collection. Where sufficient stakeholders were not willing to collaborate, sites were identified by the project team and samples were collected by them. All stakeholder information is protected by a Non-Disclosure Agreement (NDA) and stakeholder-specific details are not presented in the report. Once sufficient sample sites had been identified, sampling in the various sectors commenced. In general, sampling followed the IWUAB toolkit recommendations. Five sites in each sector (municipal, agriculture, industrial, mining) were sampled. Sampling followed the frequencies recommended by the IWUAB toolkit. In general, samples were collected from the effluent (for point source release), and the resource upstream and downstream. Samples were analysed at an accredited toxicological laboratory using tests suggested by the IWUAB toolkit. These tests used bacteria, algae, crustaceans and fish as test taxa. In all cases, test endpoints were mortality or inhibition. Test results were combined to give a hazard class, which is an ecologically meaningful classification indicating toxic hazard. This was used in assessments of compliance criteria generated by the IWUAB.

The recommendations of IWUAB user interface were assessed to examine the results in light of returned data, stakeholder input, and the literature. Several stakeholder workshops contributed to this process. Stakeholders were also trained in the use of the IWUAB toolkit and feedback was used in assessing toolkit functionality in terms of its goal in supporting the water use authorisation process in the face of identified lack of capacity at the regulator.

RESULTS AND DISCUSSION

Of the various sectors, municipal effluent was the most toxic, with lower toxicities found in effluent from the mining and industrial sectors. Effluent toxicity at each site also varied considerably with time, and ranged from effluent with a slight hazard risk to a very highly toxic effluent that caused complete mortality in all test taxa. The agricultural sector contained no sites with point effluent release that could be sampled.

A sitewise comparison of the impact of the effluent on resource hazard classification was not able to consistently identify effluent related changes to the resource toxicity hazard. Inspection of the data revealed that the impact of the effluent on the resource was modified by effluent toxicity levels, and that the resource responsiveness to effluent varied with time. An extreme of this was a site where the discharge of highly toxic effluent that caused complete mortality in all test taxa led to no change in the condition of the resource, which showed no acute hazard. The IWUAB toolkit recommends for compliance that the resource hazard class should not change by more than one hazard class unit from upstream to downstream. This was only exceeded three times, and always in the municipal sector. In all cases these occurred during the dry season. However, other sites receiving equally toxic effluent in the dry season in this sector showed no response. The change seems to be a response to low flow in a relatively small receiving water body. The agricultural sector, which received no point-released effluent, showed no increase in toxicity from upstream to downstream at any time.

Upstream toxicities, representing the site before effluent impact, were mostly in hazard class two or three (slight to moderate acute hazard). Only 6% of upstream samples collected had no detectable toxic risk. This represents an uncomfortable background level of toxicity in South African water bodies. 32% of upstream samples exceeded the hazard class proposed as a general compliance limit for downstream samples, and as such are toxic enough that more than 50% of at least one test taxon will be affected by exposure to the water. Differences between sectors in upstream toxicity were minor, with the municipal sector, located in urban areas, being on average less toxic by a very small extent. The remaining sectors had comparative upstream toxicities. No link between catchment, geographic location, or location in urban or rural areas was found to significantly modify background toxicity. However, relatively large changes in upstream toxicity at most sites negated the possibility of defining compliance requirements based on instream toxicity except in terms that relate upstream and downstream toxicities.

Downstream toxicities, with a few exceptions, are largely a function of upstream toxicities. Differences in downstream toxicity between sectors was negligible. Although no statistically significantly different changes in toxicity from upstream to downstream were found, these equivalent toxicity levels at downstream sites indicate a slightly greater average change in toxicity from upstream to downstream in the municipal sector. Average changes in the other sectors were negligible.

The blanket compliance criterion suggested by the IWUAB toolkit that the downstream toxicity be maintained at hazard class two (slight hazard) or less was found to legally indefensible in light of collected data. Upstream hazard classes, representing the un-impacted condition, of three (moderate hazard) were found in 30% of samples collected. It was the second-most common upstream hazard class encountered after class two. Given that simultaneous upstream hazard classes of three were fairly common, it is not defensible to use this as a blanket criterion for downstream hazard risk as this could easily be challenged.

The suggested criterion where upstream results at sites are compared to simultaneous downstream results, and the proposed compliance criterion is that change in hazard class from upstream to downstream should not change by more than one hazard class unit, is defensible. By indicating that the difference should be more than one hazard class unit, boundary effects, where a shift in class can be triggered by relatively small changes in toxicity test results, are avoided. This ensures that for the compliance criterion to be exceeded, a clear toxicity change will have taken place and the outcome will not be easily challenged on the basis that it may occur by chance. On the other hand, a change of two (or more) hazard classes will mean that it will require a relatively large change in toxicity for oversight to be triggered. As an example, toxicity would need to change from levels where toxicity effects are not statistically detectable, to levels where more than half, but not all, of at least one test taxon are killed. Alternately, toxicity levels would need to increase from a point where more than half, but not all, of one test taxon was killed to a point where all organisms in all tests were killed. The suggested compliance criterion seems eminently defensible as a result, but it is not highly sensitive.

A range of four different tests using representatives of the bacteria, green algae, crustaceans and fish were used to assess responses to the various samples. Responses of test taxa to the different effluents varied, and, surprisingly, differences in samples from the resource between sectors was also found. Municipal effluent was more toxic than any other across the different sectors. The endpoint of algal and bacterial tests allow for assessment of growth or bioluminescence respectively, and both showed stimulation in some samples. No test was most sensitive in all sectors, which supports the ongoing use of a range of tests in assessing effluent toxicity.

The methods used to combine a number of test results into a single class for a sample accord with previous approaches proposed for toxicological monitoring in South Africa. The method produces a simple, ecologically relevant and understandable classification that ought to be acceptable to and understood by managers. Finally, the method is capable of using screening tests, rather than definitive tests, to generate a class and thereby reduces the cost burden of testing.

The compliance criteria produced by the IWUAB toolkit are problematic at times. Within-site temporal variation rules out the simple application of criteria based on downstream toxicity as an indication of impact, as upstream toxicity may be high, and in this case, a simple criterion based on downstream toxicity without reference to simultaneous upstream toxicity will be easily challenged on a legal basis. As upstream data are collected it makes sense to use this a reference for criteria based on downstream toxicity, which the IWUAB toolkit currently does. However, use of upstream toxicity as a reference point is complicated by the fact that water bodies receiving effluent may be ephemeral, and when flow is absent at an upstream site, no reference point is available. In these cases, downstream water will generally be composed of effluent alone. As such, the only option when this occurs is to base compliance criteria on effluent or downstream toxicities. This occurred several times during the project, and was identified as a challenge to use of relative measures to define compliance criteria by stakeholders at workshops held during the project.

Some other sampling recommendations need more clarity. Point-source effluent release points are less likely to lead to challenges in sampling, and the sampling suggestions as given in the IWUAB guidelines will cover the majority of cases. The guideline does not however deal with cases where effluent from different sources is mixed prior to discharge to river. Another area that needs consideration will arise when diffuse effluent is released over a wider area. Given the narrow spatial extent required for sampling around an effluent release point, conflicts with other impacts are less likely. However, when effluent is released over a larger area, as in diffuse release, conflicting impacts are more likely and the sampling strategy in these cases needs more consideration so that compliance requirements generated relate to a single water authorisation in a defensible way.

The IWUAB toolkit collects a range of user data, but currently uses little of this to generate output. Inclusion of information on river flow could be used to generate output, and well as information related to the current state and management goals for a river would also be valuable in this regard. Finally, some consideration needs to be given to storage of this user data so as to improve the toolkit with time. Storage of test results together with the user input would also be valuable in improving the toolkit.

Effluent data is not used at all in generating compliance threshold recommendations. As noted above, where upstream flow is absent, use of effluent data may be required. Use of information on the resource will provide more defensible compliance conditions (but see above for problems with one current compliance recommendation) but consideration needs to be given to use of effluent data where it may be appropriate.

The costs of sampling as per the recommended schedules, and of undertaking analyses at accredited toxicology laboratories, may be overly burdensome for some water users. This is not likely to serious impact larger concerns, but smaller enterprises and emerging farmers may struggle to meet the demands of the recommended testing and analytic schedule. Given state identification of the latter as a national priority, this will require some consideration.

During the course of the project, it was discovered that the great majority of industrial enterprises discharge their effluent to sewer and not to surface water. This transfers the responsibility for final discharge to surface water from the effluent producer to the receiving wastewater treatment plant. The IWUAB toolkit does not make any recommendations about testing of this effluent. As effluent release to sewer is generally covered by local by-laws or other laws, regulations or agreements, it may not be appropriate for the toolkit to cover this. This project found no clear link between the proportion of industrial effluent received by wastewater treatment works and their toxic impact on receiving water, so inclusion of information on industrial influent receipt by wastewater treatment works may have no relation to anticipated effluent toxicity. However, the tests recommended for municipal and industrial effluent may need to be aligned depending on whether industrial effluent is treated.

A number of other smaller issues related to the toolkit interface are presented.

The IWUAB toolkit was presented to stakeholders from the regulator, consultants, and other people of interest at workshops early in the project, and later on when most data had been collected. A training process was offered to potential user and copies of the current version of the toolkit were distributed. The IWUAB toolkit was welcomed by stakeholders from the regulator as a new tool to improve the toxicological testing requirements attached to water use authorisation processes. However, the use of the toolkit to set licensing compliance criteria was questioned, and several stakeholders indicated they would support its adoption as a screening tool only. Other potential issues were raised by stakeholders, but these are for the most part easily addressed and are covered elsewhere in the report. DWS stakeholders stated they supported the adoption of the IWUAB toolkit provided that further engagement was undertaken to align the toolkit with DWS approaches and goals. The issue of compliance recommendations was highlighted as crucial in this regard, as compliance terms in a water use license needed to be simple, clear, unambiguous and legally defensible.

GENERAL

All but one of the aims of the project were addressed. Refining the toolkit to address the aims of the regulator will require extensive engagement with the regulator that was beyond the scope of this project. This report highlights feedback from the regulator and other stakeholders that will aid in this process. The IWUAB toolkit was presented to stakeholders from the regulator and elsewhere on several occasions, and a training workshop was undertaken. Toxicological data was collected from five sites in each of four sectors and is analysed and presented in this report. Quality was assured through the use of accredited toxicological laboratories which undergo regular quality assurance, and the use of tests that are widely accepted and standardised. Catchment limits were assessed but results from the project reveal that within-site variation in toxicity is high enough to negate the application of a single guideline over a large geographic and spatial extent. Other factors were assessed as being appropriate for the generation of general guidelines, but within-site and between-site variation was high enough to preclude this approach. Finally, the regulator indicated that adoption of the toolkit for formalized and routine application would require an engagement that is beyond the scope of this project.

CONCLUSIONS AND RECOMMENDATIONS

The IWUAB toolkit is a valuable addition that will do much to address lack of toxicological capacity at the regulator and thereby lead to the formal and widespread adoption of toxicity testing in the management of water quality in the country. The methods used for classification of toxicological output align with international practice and approaches adopted in South Africa. The toolkit produces a classification that should be acceptable to managers and is easily understood in terms of ecological relevance. Finally, the toolkit suggests compliance criteria for use in an authorisation process.

Data collected revealed that of the sectors assessed, municipal effluent was notably more toxic that others assessed. Despite this, the impact of the effluent on resource toxicity varied considerably. The toxicity of the effluent and the upstream resource varied considerably with time. No effluents were released from sites assessed from the agricultural sector. Downstream toxicity in nearly all cases was more related to upstream toxicity, and the impact of effluent was for the most part limited.

For adoption of the toolkit for routine application by DWS and CMAs, the proposed compliance recommendations are problematic. One of the suggested compliance criteria was found during the course of this project to not be legally defensible. In addition, some of the sampling guidelines offered by the IWUAB toolkit may lead to the generation of legally indefensible compliance recommendations.

In order that the toolkit be adopted to support the use of toxicological methods in water quality management and the generation of SDCs, it is imperative at this point that an engagement with appropriate representatives from the regulator be initiated. The purpose of this is ensure that all facets of the IWUAB toolkit are aligned to DWS and CMA requirements, and the compliance conditions that they generate are reasonable and legally defensible. An engagement process which enables DWS and CMAs buy-in to the toolkit and which, though continuous engagement addresses their concerns and generates a feeling of ownership, is crucial to this.

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ACRONYMS & ABBREVIATIONS

AMD	Acid Mine Drainage
CMA	Catchment Management Agency
DEEEP	Direct Estimation of Ecological Effect Potential
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
ERWAT	East Rand Water Care Company
GARL	Golder Associates Research Laboratory
HCp	Hazardous Concentration for p percent of species
IWUAB	Integrated Water Use Authorisation Bioassay (Toolkit)
NAEMP	National Aquatic Ecosystem Health Monitoring Programme
NCMP	National Chemical Monitoring Programme
NDA	Non-Disclosure Agreement
NEC	No Effect Concentration
NEMP	National Eutrophication Monitoring Programme
NFEPA	National Freshwater Ecosystem Priority Areas
NMMP	National Microbiological Monitoring Programme
NPDES	National Pollutant Discharge Elimination System
NTMP	National Toxicity Monitoring Programme
PEC	Predicted Environmental Concentration
PES	Present Ecological State
PNEC	Predicted No Effect Concentration
RDM	Resource-Directed Measures
REMP	River Eco-status Monitoring Programme
RHP	River Health Programme

RQO	Resource Quality Objectives
SANAS	South African National Accreditation System
SDC	Source-Directed Controls
SSD	Species Sensitivity Distribution
TU	Toxicity Units
TUa	Acute Toxicity Units
WET	Whole Effluent Toxicity
WMS	Water Management System
WRC	Water Research Commission
WUL	Water Use License
WWTW	Wastewater treatment works

GLOSSARY

The definitions presented here are largely drawn from Scherman et al. (2004), Chapman et al. (2011a) and Pearson et al. (2015). The terminology covered is not exhaustive and refers only to terms that are used in the current report.

Acute test	An acute effect is one that takes place in a short time (from less than 24 hours to a few days) with the exposure period being short relative to the life span of the test organism. Acute toxicological testing often uses mortality as an endpoint but it is important to note that this need not be the endpoint of an acute toxicological test. Compare with chronic test.
Chronic test	A chronic effect relates to the impact of a relatively long-term exposure to a test compound, often with the test compound at lower levels than might be expected in an acute test. Exposure is commonly for at least 10% of the life span of the test organism. The endpoint of a chronic test may relate to mortality but more commonly refers to a sub-lethal response, such as reproductive success, movement, etc. Compare with acute test.
Diffuse source or release of effluent	This occurs when effluent is not released from a spatially constrained point, but over a broader area. Diffuse release can introduce an effluent to a water body through surface runoff, baseflow, rainfall and other means. In contrast to point release, with diffuse release it is commonly difficult to determine the source of the effluent, and it is also a challenge to quantify the amount of effluent entering the system.
Ecological health	The capacity of an ecosystem to support a balanced and integrated combination of physicochemical habitat characteristics, together with biotic components, on a temporal and spatial scale that are comparable to that of the natural characteristics of the system. High ecological health implies a low level of anthropogenic impact.
End Point	Measured response or analytic target. In ecotoxicology an end point may indicate the concentration of the test compound required to affect a certain proportion of the test organisms. The effect in question may change and can refer to mortality, or reproductive success, or another measurable response.
Hazard	A state that may increase risk and lead to an undesirable condition.
Point source or release of effluent	Point source effluent release refers to a spatially concentrated release of an effluent, generally through a pipe or canal. The effluent remains at full concentration until it is released. Because of this, sampling of effluent before discharge can easily be undertaken.
Resource	A water resource, which would include surface water, estuaries, or aquifers.

Resource quality	This refers to all components of a water resource, including the quantity, pattern, timing, water level and degree of assurance of instream flow, the physical, chemical and biotic characteristics of the water, the quality of instream and riparian habitat, and the characteristics, condition and distribution of aquatic biota.
Risk	The likelihood, usually given as a probability, of a particular effect. Risk relates to the potential existence of an effect and uncertainty as regards its expression. Risk relates to the likelihood of an effect as well as its potential frequency.
Toxicity unit (acute)	The concentration of the effluent (100%) divided by the effluent concentration leading to 50% effect (here the EC_{50}) at the end of the acute exposure period.
Whole effluent toxicity	Whole effluent toxicity is the aggregate toxicity of all the compounds present in a whole, complex effluent. Whole effluent toxicity testing can assess effluent toxicity directly where chemical assessment of the effluent relies on testing for and detecting all potential toxins in the effluent, and knowing and understanding their combined impact on the test organism.

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

The current project has been undertaken in order to pilot-test the application of the Integrated Water Use Authorisation Bioassay (IWUAB) Toolkit (Pearson et al., 2015), and to train potential users of the tool in order that it can be used to facilitate inclusion of toxicological testing in Water Use Licenses (WUL) and compliance monitoring. In this way, the project aims to take a step towards formal and widespread inclusion of toxicological testing in the suite of tools used for management of South Africa's water resources. This project follows several Water Research Commission-funded initiatives that explore means to practically implement toxicity testing as an additional tool for use in management of resource quality in South African freshwater systems (e.g. Hunter et al., 1997; Haigh and Davies-Coleman, 1999; Scherman et al., 2003; Scherman et al., 2004; Slabbert, 2004; Chapman et al., 2011a, 2011b; Griffin et al., 2011; Slabbert and Murray, 2011; Pearson et al., 2015).

The South African National Water Act (NWA) (No. 36 of 1998) provides for water resource protection through implementation of the Resource Directed Measures (RDM) and Source Directed Controls (SDCs). RDMs provide quantitative and qualitative resource quality objectives (RQOs) for the quality of the water resource, while SDCs regulate the source of impact from abstraction or discharge through water use licensing (WUL) systems and other measures. These measures are used as tools to ensure adequate water quality to aquatic ecosystems in order to provide for a state of ecosystem health that will ensure sustainable use of the resource (CSIR 2010).

The NWA mandates the Minister of Water and Sanitation to establish national monitoring systems that monitor, record, assess and disseminate information on South Africa's water resources. This requirement of the Act is implemented through the National Water Resources Strategy (NWRS 2; DWA 2013), which gives direction to the development and implementation of a series of national water quality monitoring programmes. Among these programmes are the National Chemical Monitoring Programme (NCMP), the National Toxicity Monitoring Program (NTMP), the National Microbial Monitoring Programme (NMMP) and the River Health Programme (RHP, now the River Eco-status Monitoring Programme (REMP), part of the National Aquatic Ecosystem Health Monitoring Programme (NAEHMP)). The NCMP measures, assesses and reports in-stream water quality mainly by monitoring physicochemical parameters; the NTMP measures, assesses and reports on the status and trends of the nature and extent of potentially toxic substances in South African water resources (watercourses, groundwaters and estuaries) as well as the potential for toxic effects to selected organisms; the NMMP measures, assesses and reports on indicators of faecal pollution; while the RHP measured, assessed and reported on the overall ecological status of river ecosystems in South Africa. The overall objective of all these programmes is to ensure good water quality of the country's freshwater resources and to ensure ongoing sustainable water use.

Environmental water quality management uses three tools, or approaches, in managing water quality to ensure a supply of water that is adequate for the needs of human users and the environment (Palmer et al., 2005). The first, and perhaps the most obvious approach, is directly monitoring the chemical or microbial composition of water. This has the advantage of giving a simple and direct assessment of the chemical or microbial composition of the water. However, given the very wide variety of potential contaminants, direct monitoring of all or most potential contaminants is practically overwhelming and prohibitively expensive. A complementary approach is monitoring the biota in water resources (biomonitoring), which has the advantage that any impact on the biota should be detected, without any need for contaminant-specific monitoring. However, the disadvantage is that detecting an impact does not necessarily lead to an understanding of the cause thereof. It may not also be clear when the impact occurred, as recovery is not immediate (this is also an advantage as each sample reflects an extended timeframe). Straddling the middle ground between these two approaches is toxicology, where the effect of a toxin or an effluent on a particular biota is assessed in a manner that may define a dose-response relationship.

Chemical monitoring of South Africa's water resources is well developed, having been undertaken routinely since the 1980s, and earlier at some sites (Huizenga, 2011). Routine chemical monitoring assess a range of parameters including major salts and nutrients, though compounds such as heavy metals do not form part of routine monitoring (Ashton and Dabrowski, 2011; Griffin et al., 2014). Biomonitoring has taken place as part of the River Health Programme, now part of the National Aquatic Ecosystem Health Monitoring Programme, but it is of more recent provenance and as a result has produced fewer data. Toxicological testing as a tool in water management is currently undertaken on an ad hoc basis (DWA 2013; Chapman et al., 2011a). When applied, the approach uses an effects-based test where the toxicity of effluent is assessed directly. Limited testing of water in the receiving body is undertaken, as preliminary monitoring of resource quality using toxicological tests only began in 2014 (David Odusanya, DWS, pers. comm.)

Despite the application of the above monitoring programmes, water quality in South Africa is degrading (CSIR 2010). The major threats have been identified as changing pH, salinization, eutrophication and microbial contamination, with other lesser-known contaminants such as biocides and heavy metals raising concern (DWA 2011). Identified root causes of these water quality issues include effluent release from wastewater treatment works, mining, industry, and diffuse contamination from agriculture, among others.

Aquatic toxicity testing is an important management and screening tool that provides water quality information that is linked to ecological risk in the receiving ecosystem (Scherman et al., 2004). As such, aquatic toxicity testing has been used in many countries as a management option in preventing deteriorating water quality in aquatic ecosystems (Slabbert et al., 1998), whereby different test organisms are exposed to a facility's wastewater (effluent) and the effect on the organisms quantified. The results of these tests are then used to estimate concentrations of the effluent that would be able to provide adequate aquatic ecosystem protection. The effluent discharge license can then be specified in terms of a toxicity test endpoint and not only as concentrations of known toxins in effluent as provided by physicochemical assessment or as a biotic index as provided by biological assessment.

In the United States of America, the principal law governing pollution of surface waters is the Federal Water Pollution Control Act, or Clean Water Act. One way of implementing the Clean Water Act's prohibition of the discharge of toxic pollutants in toxic amounts is through Whole Effluent Toxicity (WET), which tests the aggregate toxic effect to aquatic organisms from all pollutants contained in a facility's wastewater (USEPA 2004). WET tests measure wastewater's effects on specific test organisms' ability to, amongst others, survive, grow and reproduce. WET test methods consist of exposing living aquatic organisms to various concentrations of a sample of wastewater, usually from a facility's effluent stream. WET tests are used by the National Pollutant Discharge Elimination System (NPDES) permitting authority to determine whether a facility's permit will need WET testing.

In order to improve management of water quality issues related to effluent discharge, the Department of Water Affair and Forestry (DWAF, now DWS) introduced Direct Estimation of Ecological Effect Potential (DEEEP), an approach for assessing whole effluent toxicity in effluent discharges (DWAF 2003; Slabbert, 2004). DEEEP grew from a survey of international experience (e.g. Tonkes and Baltus, 1997; Tonkes et al., 1999; Chapman, 2000; Power and Boumphrey, 2004), with methods adapted for local conditions. DEEEP comprises a range of assays assessing oxygen demand, lethal (acute) and sublethal (chronic) toxicity, bioaccumulation, mutagenicity and persistence potential of effluents, using test organisms from a range of trophic levels

(Jooste and Slabbert, 2006). DEEEP offered a promising way to manage the quality of effluent discharges, but the method was never formally adapted beyond pilot-scale (Chapman et al., 2011a).

In the following years, several projects dealing with the potential and capacity for implementing routine toxicological testing in South Africa were undertaken. Slabbert and Murray (2011) produced a tool to facilitate selection of appropriate toxicological tests as they noted a limited understanding of toxicology was hindering its adoption by the regulator. Griffin et al. (2011) produced a survey of the efficacy and cost-effectiveness of various test methods. Chapman et al. (2011a, 2011b) focused on capacity, and quality management and assurance of toxicological testing. Finally, Pearson et al. (2015) produced a survey of international practice with respect to toxicological testing, testing strategies, and finally produced and documented a toolkit to facilitate the use of toxicological methods in Water Use Licencing. The Integrated Water Use Authorisation Bioassay toolkit (IWUAB) uses information on the broad sector of the source (currently agriculture, industry, municipal, and mining), the type of effluent, ecological state of the receiving body, dilution capacity, historic toxicity and other factors to generate a sampling and analytic protocol for use in compiling terms for a Water Use License.

The tests selected by the toolkit largely follow the recommendations of DEEEP for acute testing, and the selection of tests includes a battery of acute tests covering a range of trophic levels. Samples are collected from effluent as well as upstream and downstream receiving body water (in comparison to DEEEP, where only effluent samples were tested). Sampling strategy and timing is also addressed by the IWUAB. It is currently limited to testing of fresh water only, but has the potential to be expanded to cover estuarine or marine samples, and to cover testing of sediment as well.

The IWUAB toolkit was produced to support Water Use License applications, although it is able to provide bioassay methodological input to clients and to consultants in other contexts (Pearson et al., 2015). The format and nature of the IWUAB toolkit derives from workshops with various stakeholders at which their needs regarding the use of bioassays in the Water Use Licensing processing were explored. These were used to compile a list of toolkit inputs and outputs, and were included in the toolkit along with supplemental information to support adoption of the process. Accompanying documentation (Pearson et al., 2015) contains further information on why toxicity testing is an important part of the licensing process, as well as supporting documentation explaining how to use the toolkit and how to interpret and implement the licensing conditions generated as toolkit output.

This project tests the application of the IWUAB toolkit in toxicity testing in the four sectors currently addressed by the toolkit to evaluate the testing regime, and to evaluate the results from the four sectors with the aim of refining the toolkit for practical implementation of toxicological testing in South Africa. The project will also to undertake a public engagement process to present the IWUAB toolkit to potential end users.

1.2 PROJECT AIMS

The aims of the project follow:

1. To demonstrate the application and implementation of the Integrated Water Use Authorisation Bioassay (IWUAB) Toolkit in a number of locations and industries.

2. To develop and build capacity on the use of the developed system and aquatic toxicity testing in general.

3. To collect toxicological data over one hydrological year and to prepare the reports that will enhance the work of the regulator in assessing compliance/non-compliance.

4. To highlight quality assurance practices associated with aquatic toxicity testing.

5. To set catchments-based limits in selected catchments through stakeholder engagement and dialogue.

6. To refine the developed decision support system (IWUAB Toolkit) for potential application by DWS and CMAs for water use authorisation.

1.3 SCOPE AND LIMITATIONS

The current project has the focus of assessing the IWUAB toolkit with the aim of finalizing the toolkit so as to support implementation of toxicological testing of effluent emissions as a tool in water quality management in South Africa. The project does not aim to undertake a statistically meaningful survey of all effluents from all sectors in South Africa, nor does it aim to provide a statistically valid toxicological survey of water bodies in South Africa. It is acknowledged that effluent from all representatives of a sector may vary considerably, and also that different resources may respond differently to receipt of effluent. As such, the project does not claim representatively of all potential emitters, or all potential resource responses.

CHAPTER 2: TOXICOLOGICAL RESULTS FROM FOUR SECTORS

2.1 INTRODUCTION

The IWUAB toolkit was produced as part of ongoing research that aims to implement appropriate toxicity testing of effluent in order to improve management of water quality in South African surface water. Production of the toolkit was spurred by indications that one factor limiting adoption and use of toxicological testing in South Africa in the water use authorisation process was lack of toxicological capacity at the regulator which hinders appropriate application of testing and interpretation of results (Pearson et al., 2015). The toolkit aims to simplify test selection by recommending tests after input of site and emitter details. The toolkit also proposes use of the Persoone et al. (2003) toxicological result classification scheme to produce output ranked into classes that will easily be understood by users, and generates compliance criteria recommendations.

In order that the potential of the IWUAB toolkit may be assessed, it was necessary to assess the toolkit's performance in a pilot study. This chapter reports on a pilot assessment of toolkit test recommendations. The results of testing are assessed in the light of toolkit recommendations, and the performance of the toolkit in producing appropriate testing recommendations is also assessed.

Sampling and testing recommendations produced by the IWUAB toolkit were largely based on professional experience dealing with effluents. The recommendations differ depending on which sector the effluent derives from. Sectors that were implemented in the IWUAB toolkit at time of testing included the municipal, industrial, mining and agricultural sectors. These were the sectors that were used during this assessment of results from sites in the various sectors. The IWUAB toolkit has the potential to expand to include other identified sectors where it is warranted or required.

The results of this project will inform refinement of the toolkit and feed back to the developers in order that a practical and realistic aid is available to support the use of toxicological testing in water quality management.

In order that a cost-effective approach to sampling is taken, it was desirable that the project align itself with partners in the four industrial sectors in order to ensure access to effluent sources and rivers that might be on private land, and to facilitate understanding of effluent streams and potential temporal changes therein. Collaboration with partners in this way in some cases extended to the partners managing the sampling and shipment of samples to accredited toxicological facilities and thereby reduce expenditure on travel and increase the funds available for sampling. Partners that required legally binding non-disclosure agreements (NDA) received them. Non-disclosure agreements included the proviso that site and partner identities be masked in order that partners and release points could not be identified. For this reason, partner and site identities were assigned a unique randomized numeric identifier, and these will be used throughout this report. All information that may identify a site, including the name of the river the site discharges to, is held in a password-protected encrypted file, and will not be revealed in this report. Non-location specific information, such as river ecological condition, is not protected in this way and may be used.

2.2 METHODS

2.2.1 Sampling

Sampling was undertaken in all sectors, producing annual datasets from five locations each in the industrial, agricultural, municipal and mining sectors. Where possible, samples were collected by collaborating organisations in the four sectors. Sample collection in the industrial sector was complicated by difficulties in identifying industries discharging directly to river, as nearly all industries that were willing to collaborate discharged effluent to sewer rather than to surface water. A result of this practice was that the majority of industrial effluent discharged to the resource was emitted from wastewater treatment works, and was therefore classified as deriving from the municipal sector. For this reason, industrial samples were collected directly from two sites that discharged to surface water, and also from three wastewater treatment works (23713 63 Ml.day^{-1}, 35546 16 Ml.day^{-1}, 84187 105 Ml.day^{-1} design capacity) with a significant industrial input (30-40% of influent). Agricultural samples were collected from four sites under year-round irrigation with significant crop densities, and one cattle feedlot. Mining samples were collected from gold and platinum mines. Municipal samples were collected from wastewater treatment works with design capacities ranging from 1-160 Ml.day^{-1}, and varying loads of industrial influent (43114 8 Ml.day^{-1}, 49909 63 Ml.day^{-1}, 64033 1 Ml.day^{-1}, 71056 155 Ml.day^{-1}, 75130 14 Ml.day^{-1}). All sites bar two were located in and around Gauteng, in Gauteng and the North West Province. The remaining sites were in the Eastern Cape. Sampling frequencies were largely based on IWUAB output and are presented in Table 1. In all cases, samples were collected for one year after initiating sampling.

Table 1: List of tests undertaken and sampling frequencies in the four sectors assessed.

	Municipal	**Mining**	**Agriculture**	**Industry**
Vibrio fischeri bioluminescent test: EN ISO 11348-3 (2007)	×	×	×	×
Selenastrum capricornutum growth inhibition test: OECD Guideline 201 (2006)	×	×	×	×
Daphnia pulex acute toxicity test: US EPA (2002)	×	×	×	×
Poecilia reticulata acute toxicity test: US EPA (1996)		×	×	×
Frequency (year^{-1})	6	4	4	4

As per the collaboration agreements signed, neither the identity of specific collaborators nor the location of sites sampled will be revealed in this report. Methods for sampling and analysis follow the output of the IWUAB toolkit for generalized sites in each sector. Test selection and sampling frequency generally followed the IWUAB recommendations (Table 1). Samples from the municipal sector were assessed using the fish test on several occasions to provide comparative data. At each site with a point release of effluent (here mining, municipal and industrial sectors), a sample was collected from the effluent stream, and two points (one upstream and one downstream and within 5 km of the effluent release point) in the receiving water body. The samples were delivered to a SANAS accredited toxicology laboratory, where the tests for that sector were undertaken. For routine testing, the IWUAB recommendations are that all tests should be screening tests on undiluted samples. When acute toxicity in a sample is detected (defined as an effect greater than 50%), the IWUAB output recommends that a definitive test should be used, "based on best professional judgement". For the purposes of the project, it was decided to use screening tests of samples from receiving water bodies, and definitive tests for effluent samples.

Samples collected in the agricultural sector were not collected from point-released effluents as no point release of effluent was present. Instead, the samples were collected upstream and downstream of the identified impacts. The samples from these points were assessed using screening tests on undiluted receiving water. Output from the IWUAB toolkit recommends sampling before and after seasonal events. As none were known it was decided to sample this sector four times per year to assess such seasonal variation as might occur.

For each test undertaken, the Acute Toxicity Units (TUa) were calculated following Tonkes and Baltus (1997) (i.e. TUa = $100/LC_{50}$) to provide a means of comparing the results of tests undertaken. The overall toxicity risk posed by the sample was calculated following Persoone et al. (2003), which calculates two indices of overall toxicity: the Hazard Class (Table 2), which is ranked from 1 to 5 and expresses the extent of toxicity in at least one of the tests, where 1 and 5 represent lowest and highest classes, respectively; and the Weight Score, which indicates how well the results are supported by all the tests undertaken and therefore the overall toxic hazard of the sample. The hazard class is used in the output related to compliance in the IWUAB recommendations, and is commonly expressed as the requirement that there should not be a decrease in the hazard class of more than one unit between the upstream and the downstream sites in the receiving water body, and that the hazard class at the downstream site should be less than three. As the hazard class is made up of classes related to percentage effect, the hazard class scores may be compared with South African ecological classifications (e.g. see Kleynhans and Louw, 2008). The class weight score is not included in any output from the IWUAB.

Table 2: Description of hazard classes (from Persoone et al., 2003).

Class	Description
Class 1	No acute hazard-none of the tests shows a toxic effect (i.e. an effect value significantly higher than that in the controls).
Class 2	Slight acute hazard-a statistically significant ($P<0.05$) PE is reached in at least one test, but the effect level is below 50%. For pragmatic reasons the 20% effect level can also be taken as the lowest PE considered to have a significant toxic impact.
Class 3	Acute hazard-the PE_{50} is reached or exceeded in at least one test, but the effect level is below 100%.
Class 4	High acute hazard-the PE_{100} is reached in at least one test.
Class 5	Very high acute hazard-the PE_{100} is reached in all the tests.

In order that the practical experience of application of testing be best simulated, all testing was undertaken by a commercial SANAS accredited toxicological laboratory (Golder Associates Research Laboratory (GARL)). The use of an accredited laboratory addresses the issues of quality assurance highlighted by Chapman et al. (2011a, 2011b) and included in the project aims.

2.2.2 Analysis

A simple graphical analysis was undertaken where hazard classes for each sample in effluent, upstream and downstream locations was undertaken and the results are presented here. Hazard classes rather than individual test results received the majority of analytic focus as these are the IWUAB's recommended method for classifying the toxicity risk as revealed by multiple tests, and they underlie IWUAB's water use license recommendations.

Several statistical analyses were undertaken to assess the results to the following questions:

Does effluent or resource toxicity vary significantly between sectors?

Do effluents from various sectors have a different impact on the toxicity of the receiving water body?

Do the different individual tests give different results in different sectors?

A simple analysis of variance following appropriate transformation (Box and Cox, 1964) was undertaken to assess the difference in toxicity (as measured by the hazard class) between sectors. This analysis was repeated assessing the hazard class of the upstream resource between sectors to determine whether effluents were discharged to rivers with a differing resource quality. A pairwise generalized linear mixed effect model with Wald chi-squared analysis of deviance on fixed effects was undertaken to determine whether upstream-downstream hazard classes varied between sectors (Zuur et al., 2009). Finally, the results of the individual tests were compared using a simple linear model to determine whether certain tests might be more appropriate for particular sectors. Prior to undertaking this test, test results were standardized and rescaled so that the extent of percentage inhibition (or mortality) or stimulation resulting from exposure to undiluted effluent was assessed. Post-hoc tests, where used, used Tukey's all-pair comparisons.

Analysis of variance was undertaken using R 3.4.1 (R Core Team 2017) and the generalized linear mixed effect model used the package lme4 (Bates et al., 2013). Plots were produced using ggplot2 (Wickham, 2009). Other R packages used include agricolae (de Mendiburu, 2017), RODBC (Ripley and Lapsley, 2017), reshape2 (Wickham, 2007), plyr (Wickham, 2011) car (Fox and Weisberg, 2011), MASS (Venables and Ripley, 2002) and grid (R Core Team, 2017).

2.3 RESULTS

2.3.1 Mining

Hazard classes of effluent at five sampling points in the mining sector are presented in Figure 1. IWUAB-generated license conditions in this sector in general agree that at no point should the downstream hazard class be three or more, and that there should not be an increase in hazard class from upstream to downstream that is greater than one class unit. An inspection of Figure 1 reveals that no site had a downstream hazard class of two or more hazard class units more toxic than the matching upstream site, and that, for the most part, there was no change in hazard class between upstream and downstream sites. However, several sites had downstream hazard class ratings of three.

Downstream hazard classes of three were found six times. In three of these, the downstream hazard class matched the upstream hazard class, and there is no evidence that the effluent had any effect on receiving water toxicity. In one, a downstream hazard class of three occurred when no upstream flow was present, and the downstream flow was comprised entirely of effluent. In two cases, downstream toxicity was one unit class greater than upstream toxicity. At site 91444, this occurred despite the effluent being no more toxic than upstream flow. At site 27186, effluent had a hazard class of four, which was the most toxic of all mining effluent assessed and it seems the effluent caused the increase in toxicity from upstream to downstream. In contrast, hazard class at downstream sites was lower that at upstream sites on four occasions. In three of these, the effluent was less toxic than upstream water and may have caused the decrease. In the last one, the effluent and the upstream water were equally toxic.

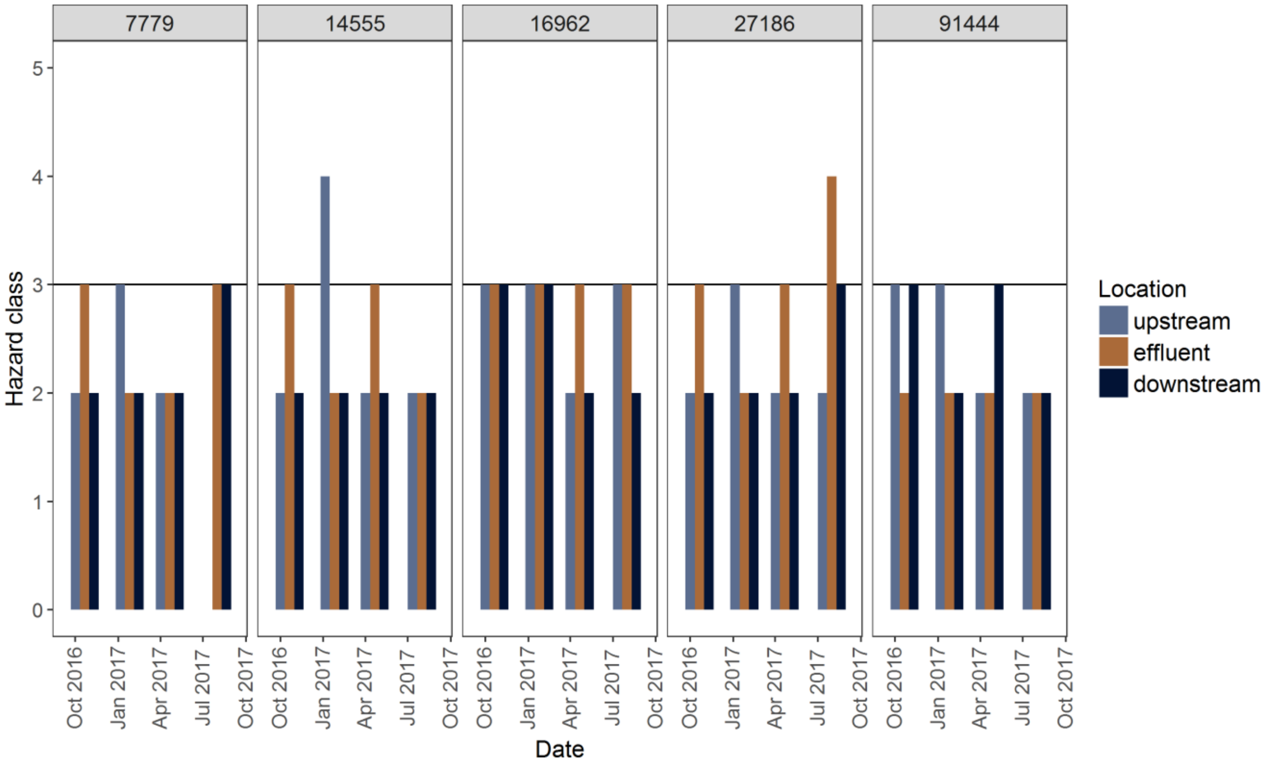

Figure 1: Hazard classes in mining effluent and in upstream and downstream samples in the receiving water body. Data are grouped by sampling point. The line indicates hazard class three for comparison with IWUAB toolkit compliance criteria.

Effluent hazard classes in this sector ranged from an average of 2 to 3, or slight to moderate toxicity. At two sites, the toxicity of the effluent was comparable with that of the receiving water. At upstream sites, average hazard classes ranged from 2.25 to 2.75, or slight toxicity, and at downstream sites average hazard classes at sites ranged from 2.0 to 2.5. The figures suggest that the impact of the effluents assessed in this sector on the receiving water was negligible.

2.3.2 Municipal

The hazard class of effluent and upstream and downstream points in the receiving water body owing to discharge of municipal effluent at five sites is presented in Figure 2. IWUAB-generated license conditions in this sector in general agree that at no point should the downstream hazard class be three or more, and that there should not be an increase in hazard class from upstream to downstream that is greater than one class unit. Three major conclusions are apparent from the plot: municipal effluent was frequently very toxic; the toxicity of effluent at any particular sampling point was not consistent over time; and the impact of effluent toxicity on the receiving body was often less than might be expected.

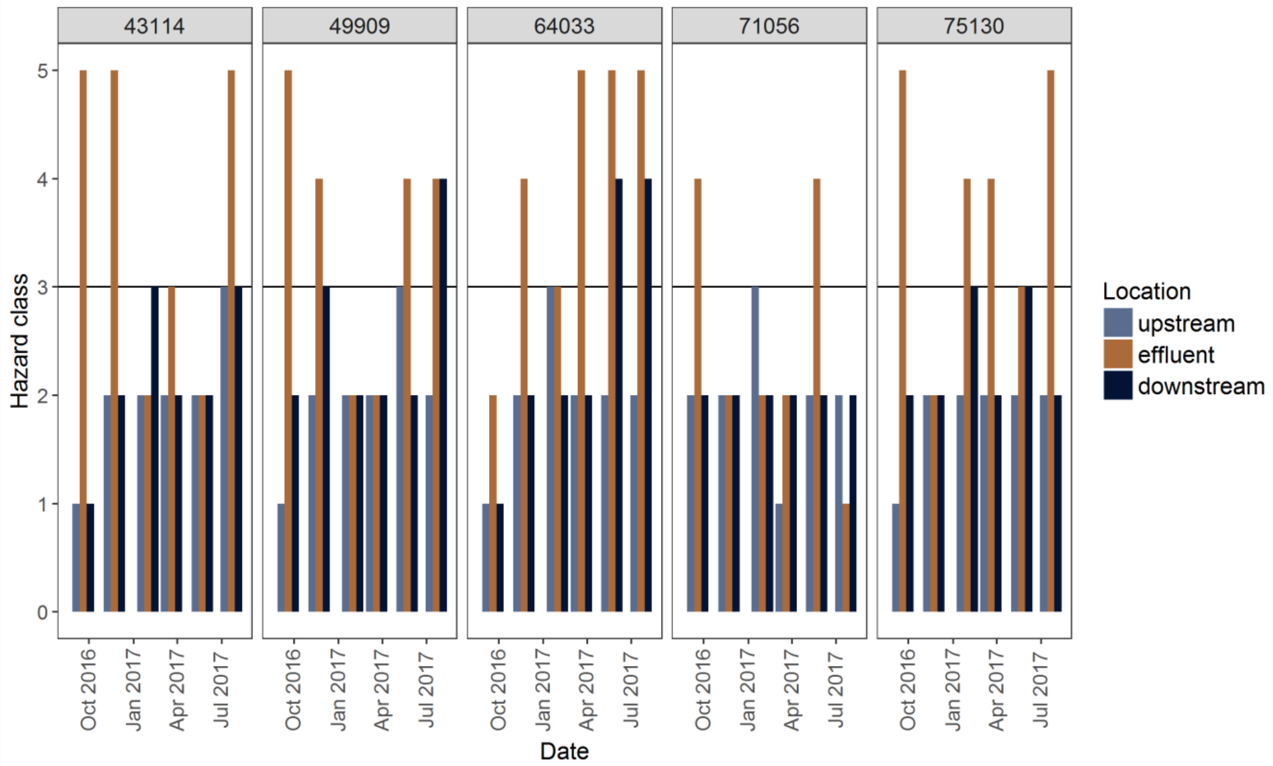

Figure 2: Hazard classes in municipal (WWTW) effluent and in upstream and downstream samples in the receiving water body. Data are grouped by sampling point. * indicates a hazard class change from upstream to downstream of two hazard class units, and the line indicates hazard class three for comparison with IWUAB toolkit compliance criteria.

Effluents in this sector were notably toxic, with considerable variation with time at each site. Average hazard class of effluents at each sampling point ranged from 2.5 to 4.0, or slightly to highly toxic. The majority of sampling points had mean effluent hazard classes between 3.5 and 4.0. Effluent toxicity at each site ranged from 2 to 5 (four sites) or 1 to 4, which illustrates that the effluent stream from a single plant can change dramatically and unpredictably with time. Dr B Shaddock (Project Steering Committee meeting November 2017) indicated the possibility that toxicity in municipal effluent (and its variability) may result from inconsistent application of chlorine to treated effluent, and the same point arose at stakeholder meetings.

The water receiving these effluents had far lower toxicity. Mean hazard classes per site from upstream receiving water ranged from 1.8 to 2.0, and at downstream sites from 2.0 to 2.5. This shift is relatively small given the high toxicities commonly found in the effluent.

A two or more hazard class units increase in toxicity from upstream to downstream receiving water would contravene the proposed water use license conditions. This occurred three times at municipal sites: twice at site 64033 and once at 49909. In the two cases from site 64033 the effluent was very highly toxic at hazard class five, while at site 49909 the effluent was highly toxic at hazard class four. At the latter site, effluent of hazard class five occurred once during the monitored period, where it resulted in a smaller increase in hazard class from upstream to downstream. At sites 43114, 64033 and 75130, effluents of hazard class five had no detectable impact on receiving water body toxicity during some sampling times.

Increases from upstream to downstream of one hazard class unit were found at all sampling sites. The frequency of this occurring ranged from once at sites 43114 and 71056 to four times at site 49909. Clear association of a one hazard class unit increase with effluent toxicity was not at always clear. At sites 75130, 49909, and 71056 this link was most notable, and increases were associated with an effluent that was more toxic than upstream water. However, the degree of toxicity of effluent seems to have played little role in changes to water body toxicity as these changes result with effluent of hazard classes two to five. At site 43114, effluent of hazard class five was found three times without changing the toxicity of the receiving water. The same occurred at sites 64033 and 75130. In contrast, decreases in toxicity were only found twice, at sites 49909 and 64033.

2.3.3 Agriculture

The results of toxicity tests of upstream and downstream sites in the agricultural sector are presented in Figure 3. No effluent samples are presented as none of the sites discharged effluent directly to the receiving water body, and IWUAB licensing recommendations for this sector indicate that upstream and downstream samples are sufficient where no effluent is produced. IWUAB licensing recommendations for this sector also recommend that the hazard class should not increase by more than one unit from upstream to downstream, and that the downstream hazard class should be less than hazard class three.

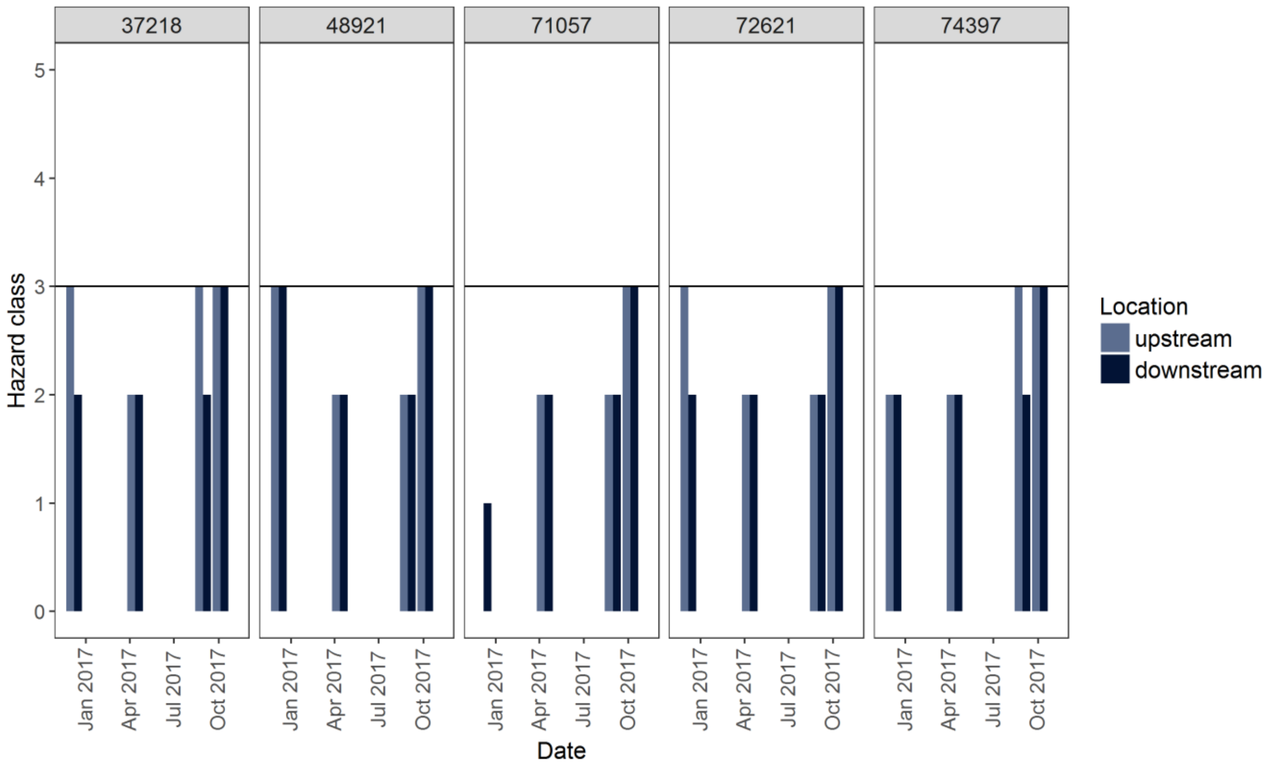

Figure 3: Hazard classes in upstream and downstream receiving water bodies at sites in the agricultural sector. Data are grouped by sampling point. No data were available for site 71057 upstream sample from December 2016. The line indicates hazard class three for comparison with IWUAB toolkit compliance criteria.

No increase in toxicity was found in any of the sample agricultural sites assessed. Locations include four sites where the receiving water was flanked by irrigated agriculture (crops varied) and one where the river flowed past a cattle feedlot. The majority of sites showed no change in toxicity between the upstream site, and on four occasions a decrease in toxicity of one hazard class unit was encountered.

Although no increase in toxicity at agricultural sites was found, all sites contravened the license conditions proposed by IWUAB output, in that downstream hazard class was three on at least one sampling occasion. On all occasions this was a result of upstream water also having a hazard class of three, with no demonstrated impact of the farm that was sampled. On four occasions, at three of the sampled sites, water toxicity improved by one hazard class unit during its passage past the sampled farm. This further emphasizes the observation that no toxic impact owing to irrigated farming or feedlotting was detected during the course of this research.

With one exception, all samples collected from this sector had a hazard class rating of two to three. Per site average toxicities of 1.8 to 2.8 were found at upstream sites, and 2.3 to 2.5 at downstream sites. As such sites in this sector would be classified as slightly to moderately toxic.

This research programme was not designed as a national survey of toxicity in aquatic bodies. Nevertheless, it is notable that despite the relative remoteness of the sites that were surveyed, no site had negligible toxicity at upstream sites. Although the sampled sites could not be linked to increased toxicity during the research period, unknown impacts led to upstream toxicities of hazard class three or less, or moderate acute toxicity at worst. Only one sample that was collected from site 71057 was found to have no acute toxicity, or a hazard class of one. The source of the upstream toxicity could not be determined during the course of the project, but is disturbing that these data suggest an at best slight acute hazard as a baseline for rivers in the sampled area.

2.3.4 Industry

Owing to a lack of willing stakeholder participation, sampling in this sector commenced later than other sectors. The samples were collected from two identified industries discharging to river in the Eastern Cape, and the effluent from three wastewater treatment works treating a large load of wastewater from industries in the Gauteng region (30-40% of influent from industry). The latter sampling strategy was adopted after queries about industrial effluent in several WMA revealed that the great majority of industries producing effluent discharge to sewer and not directly to a receiving water body. The results of sampling in this sector are presented in Figure 4. Depending on how IWUAB is applied (different industries, rivers, etc.) a range of license conditions might be recommended. Here, four samples were collected per year from each of the sites. The composite IWUAB license conditions selected for this sector were that downstream toxicity should not increase by more than one hazard class (compared to upstream toxicity) and that the downstream hazard class should not reach three or greater.

Effluent hazard classes ranged from two to four, or slight to high toxicities. Compared to results from the municipal sector, no very toxic effluents were found in samples from this sector during the research period. Average per-site effluent toxicities varied from 2.5 to 3.0. Effluent of hazard class four was collected on four occasions from four of five sample sites, and this level of toxicity did not occur regularly at the sampled sites. When effluent of hazard class four was collected, it was not on any occasion associated with increased receiving water body toxicity. On three of these occasions discharge of class four effluent had no detectable impact on receiving water toxicity, and in the remaining instance, discharge of hazard class four effluent was associated with a drop in toxicity from hazard class three to two from upstream to downstream.

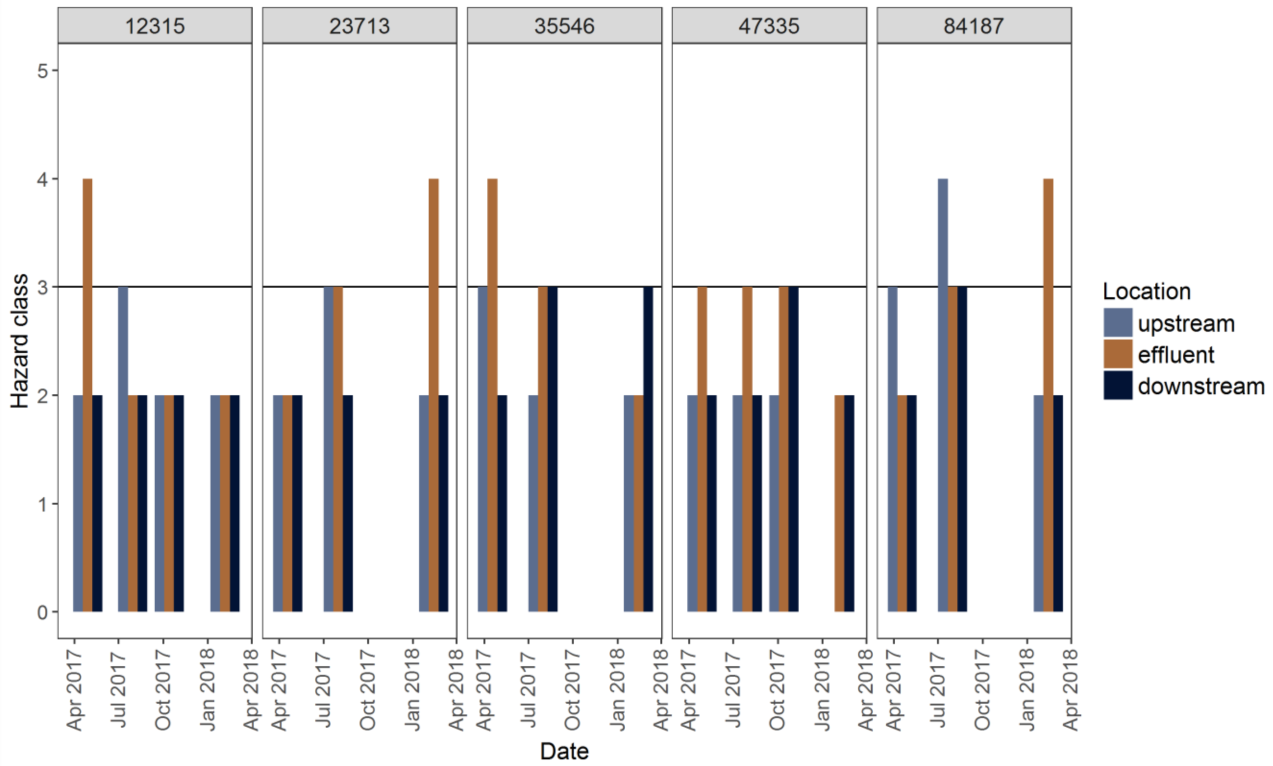

Figure 4: Hazard classes in industrial effluent and in upstream and downstream samples in the receiving water body. Data are grouped by sampling point. Samples could not be collected from 23713, 35546 and 84187 during October 2017. A lack of upstream receiving water body at 47335 precluded collection of an upstream sample during January 2018. The line indicates hazard class three for comparison with IWUAB toolkit compliance criteria.

IWUAB-proposed licensing conditions state that the hazard class for the receiving water should not increase by two class units, and in samples from this sector that never occurred. Increases of hazard class from upstream to downstream by one hazard class unit were found on three occasions from two sites. In two of these cases the effluent was more toxic than upstream receiving water, and in the last the upstream water and effluent were equally toxic. In contract, decreases in toxicity from upstream to downstream by one hazard class occurred five times at four sample sites.

The other IWUAB-proposed licence condition is that downstream receiving water body should not drop to hazard class three or worse. In the samples collected from the industrial sector this occurred four times from three sample sites. In one of these, the upstream hazard class was four, and elevated downstream toxicity can be explained by this alone. In the remaining three cases, upstream toxicities of hazard class two were increased to three after discharge of effluent. This occurred even when effluent toxicities were in hazard class two.

2.3.5 Comparisons between sectors

The toxicity of effluents varied significantly in the sectors where effluent was available (p=0.025). The mean hazard class of the effluents ranged from 2.6 (mining), through 2.8 (industry), to 3.5 (municipal). Of the data assessed, effluent toxicity from the mining and industrial sectors was comparable, and posed a slight to moderate acute hazard. Effluent from the municipal sector was more toxic and posed an on average moderate to high acute hazard. No effluent from the agricultural sector was available for comparison.

The analysis was repeated on class weight scores to assess whether support for hazard class scores varied between sectors. The results indicate that sectoral variation in class weight scores was marginally non-significant (p=0.074). Class weight score support was greater for the municipal sector (66%) than the industrial (52%) or the mining (53%) sectors.

The variation in hazard class between upstream receiving water was assessed to determine whether resource toxicity varied between sector, and by inference, the potential impact of activity on the resource. Receiving water hazard class was found to vary significantly between sectors (p=0.003). The mean receiving water body toxicity was comparable in the agricultural (2.5), industrial (2.4) and mining (2.5) sectors, and lower in the municipal (2.0) sector. All would be classed as slight to moderate acute hazard. The implication of the effluent and receiving water assessments is that municipal effluent, which was the most toxic, was discharged to the least toxic water bodies. However, it is important to note that despite the significance of the result, the difference in hazard class is relatively small. It should also be noted that the sampling strategy underlying these analyses was implemented to pilot-test the IWUAB toolkit and not to survey toxicity on a national scale, and these results cannot in any way be used to draw conclusions about toxicities of effluent and receiving water except in the context of this research.

The effect of the effluent on receiving water body quality (as in hazard class of upstream and downstream water resources) in a site-date pairwise fashion was also assessed. This analysis assessed whether, given the upstream hazard class at a particular site and sampling date, any sector had undue influence over downstream hazard class classifications, and, by inference, whether any sector had a greater impact on receiving water toxicity. No significant difference between sectors in impact on receiving water body toxicity was found, despite the finding that effluent toxicity varied significantly, as did that of the receiving water body. Assessment of the data presented in Figure 1, Figure 2, Figure 3 and Figure 4 reveals that despite occasional high effluent toxicities, impact on the receiving water was in general limited, and that resource toxicity upstream was commonly the same as resource toxicity downstream, or only one hazard class different. This may be a function of variation in the size of the water body receiving effluent leading to varied dilution rates and contributing to a more variable effect on resource toxicity. It may also be a function of variation in effluent toxicity.

2.3.6 Individual test results

2.3.6.1 Screening-Inhibition and stimulation

The data analysed thus far have all been hazard class scores, where the results of tests on several taxa were pooled in a ranked system that indicates the extent of the overall risk of toxic impact. These data have received the focus because the IWUAB recommendations are always couched in these terms, and individual test results are not considered directly when setting out compliance limits for a water use license. Nevertheless, it would add value to an assessment of the method if the results of individual tests were assessed in light of IWUAB recommendations. The tests recommended by the IWUAB included representatives of the green microalgae, bacteria, crustaceans and fishes, and are given in full in Table 1. Results from screening tests on receiving water show inhibition or stimulation in those tests. The results from definitive tests on samples show the inhibition or stimulation in undiluted samples, which is equivalent to the screening results. The test results in the four sectors are presented in Figure 5 and in Appendix B.

Inspection of Figure 5 reveals that test organism responses to effluents from the various sectors varied considerably. One notable difference between the tests is that algae occasionally showed increased mean growth or stimulation in instream sites. This is likely due to increased growth in the presence of nutrients as well as limited toxicity. Bacteria also showed stimulated bioluminescence on occasions. There was no stimulation of growth in crustaceans or fish as the test endpoint precluded this.

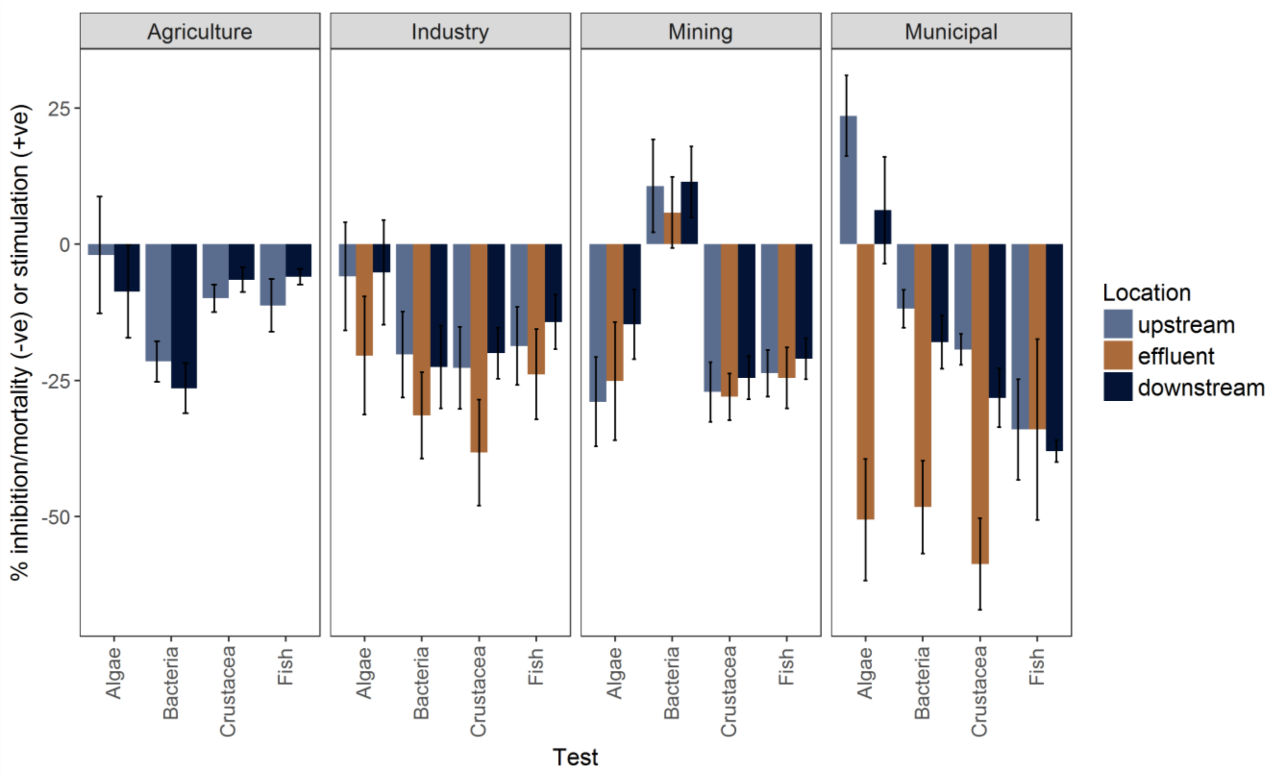

Figure 5: The results of individual toxicity tests by sector at upstream and downstream sites and in effluent. The results show the percentage effect as inhibition/mortality or stimulation (only possible for bacteria and algae) in 100% effluent or receiving water. Plots show means ± standard error for agriculture (20 agricultural, industrial, and mining samples; 30 municipal samples (fish test 5 samples); 1 test per test taxon).

The reason for differences in stimulation of growth or bioluminescence between sectors, particularly in upstream receiving water bodies or where no effluent was present, is not known. Sectoral differences in responses to effluent and downstream receiving water may be attributed to sectoral qualities of the effluent. The same cannot be said for upstream water. Nevertheless, differences in upstream receiving water toxicity are clearly apparent in Figure 5. As noted in section 2.3.5 above, some variation in receiving water body upstream toxicity was found, and this is apparent in the results presented in Figure 5. In general, receiving water before discharge of effluent had some toxic impact on test organisms, and this varied between test organisms. Where receiving water was more toxic, the impact on the various test organisms varied. The reason for this is not known, as no criteria for test site location were applied, and sites are in general scattered around Johannesburg, with more sites in the south. Agricultural sites are often more remote from built-up areas, which may account for the lower toxicity often found there. Mining sites are relatively clustered to south-west of the region. That the toxicity in receiving water is significant and varies by sector strongly supports the approach taken by the IWUAB toolkit where instream toxicity is comparative to non-impacted upstream sites, and where some of the recommendations are explicitly in terms of changes to receiving water body hazard class.

An unexpected trend noted in the mining sector was the general decrease in toxicity at downstream sites compared to upstream ones. Though not statistically significant, this trend applied across all tests undertaken. The implications of this seem to be that the resource was significantly impacted prior to effluent discharge, and that effluent discharge may have marginally decreased toxicity levels. The results in Figure 5 reveal that the effluent generally had a similar toxicity as the receiving water. Bacterial tests were the least sensitive test in this sector, followed, to a considerably lesser degree, by algal tests. The receiving water bodies at mining sites were mostly small streams, often located in close proximity to other impacts. NFEPA river classifications for these streams ranged from D to E/F, or largely to critically modified (Nel et al., 2011a).

The toxicological impact of the agricultural sector was also small. Receiving water at these sites was in general less toxic than in other sectors, and the difference between results from upstream and downstream sites was minimal. Although not significant, some negative impacts were experienced by algae and bacteria, while crustaceans and fish indicate slightly decreased toxicity at downstream sites. None of the sites in this sector discharged effluent and these results reflect the impact of diffuse discharges from irrigated agriculture and one feedlot, and any other cumulative impacts along a stretch of river (though none were known). It is not known whether any pesticide or herbicide application, or fertilization, took place between sampling events. Water body size in this sector ranged from small to medium. The NFEPA river classifications for these streams and rivers ranged from C to E/F, or moderately to critically modified (Nel et al., 2011a).

In samples from the industrial sector, results were somewhat mixed and inconclusive as to impact of effluent on the resource. Effluent samples were noticeably more toxic than receiving water to all test taxa. All receiving water was toxic to some extent to all test organisms. Effluent discharge had no detectable effect on the impact of receiving water to algae. Effluent discharge had a slight but non-significant negative impact on receiving water according to bacteria, while crustaceans and fish indicate a slight but non-significant improvement in receiving water toxicity downstream of effluent discharge. Overall, despite clear effluent toxicity, the effects on the receiving water bodies were small and inconclusive. As with the agricultural sector, water body size ranged from small to medium. NFEPA river classifications for these streams and rivers ranged from C to D, or moderately to largely modified (Nel et al., 2011a).

Municipal instream samples had profoundly different effects on test organisms. These differences were partially a function of receiving water body toxicity, and the differing responses of the test organisms to that water. Overall, tests indicate that municipal effluent was the most toxic of all sectors, with most tests showing high toxicity, although fish seemed less sensitive to the effluent but more sensitive to receiving water. Uniquely, downstream receiving water in this sector was always more toxic than upstream water (even though this may have been considerably toxic). Despite the general resource toxicity, algal growth was improved, and the effect of the effluent was to reduce this growth spurt. As before, receiving water body size ranged from small to medium. NFEPA river classifications for these streams and rivers ranged from D to E/F, or largely to critically modified (Nel et al., 2011a).

Statistical assessment of the results from the different tests in the four sectors found that differences between test results were highly significant ($p<0.001$). This is regardless of whether the test was used to assess instream water or incoming effluent, and therefore suggests that some tests are better suited for use than others. However, as was found with hazard class results, variation in data is relatively high, and it appears that resource and effluent toxicity had high variation. This reduces the power of the test to discriminate between different test and sector combinations.

The analyses found that some tests were relatively insensitive in particular sectors, and the routine application of these tests does not contribute much to the detection of toxicity, but rather illustrates that the toxic effect of the sample does not affect all test taxa. The statistically indistinguishable group of relatively insensitive tests includes algae, crustaceans and fish for agriculture, bacteria for mining, algae for municipal systems (despite the high response to effluent seen in Figure 5), and algae for industry. These results largely confirm the trends apparent in Figure 5.

In a similar light, the analysis indicated which tests were particularly sensitive for samples from particular sectors. This group represent tests that should be used in samples from these sectors in order that toxicity is detected. In decreasing order from the most sensitive, these tests include: crustacean tests in the municipal sector, fish tests in the municipal sector, crustacean tests in the industrial sector, crustacean tests in the mining sector, bacterial tests in the municipal sector, bacterial tests in the industrial sector, bacterial tests in the agricultural sector, fish tests in the mining sector, algal tests in the mining sector, and fish tests in the bacterial sector.

As noted above, variation in the data is high which limits clear statistical analysis. This variation may also have arisen from variation between responses to effluent and instream samples, as was common in tests from the municipal sector. A consequence of that is that the analysis identifies algae as less sensitive in the municipal sector. Inspection of data from Figure 5 reveals that algae were highly sensitive to municipal effluent, and, while showing growth stimulation in instream samples, stimulation was significantly decreased from upstream to downstream, indicating an instream response to effluent release. Algae do therefore respond to municipal effluent, even if the growth stimulation in upstream water helps mask this.

The group of more sensitive tests contains several where crustacean tests are the notably sensitive (municipal, industrial and mining sectors) along with bacterial tests (municipal, industrial and agricultural sectors). These tests, using very different test taxa and with different endpoints, seem to be useful general tests that might be included initially in many monitoring programmes, with the proviso that they could be removed from the programme depending on ongoing results (the relative insensitivity of the bacterial test when applied here in the mining sector, and that of the crustacean test in the agricultural sector, illustrate this).

Toxicological monitoring in the agricultural sector is complicated by the insensitivity of most tests applied here to samples from that sector. This may simply indicate the relatively good condition of the resource where samples were collected. The bacterial test was the most sensitive in this sector. The algal test was less sensitive, but showed an overall change from upstream to downstream. Neither the crustacean or fish tests were particularly sensitive. From the results presented here, it is clear that bacteria should be part of the suite of tests in use in this sector. Other tests are more difficult to recommend. Given the potential of pesticide, herbicide and antibiotic contamination from some agricultural operations, it may be best to use at least the algal, bacterial and crustacean tests at a minimum for the agricultural sector.

While this analysis assesses the response of the different tests and test taxa in the various sectors, and is able to make tentative recommendations as to the suitability of tests for toxicological testing, it must be stressed that these recommendations are based on a limited data set and as such may not apply in all circumstances. Five sampling sites per sector, nearly all in or near Gauteng, cannot represent the potential impact of all operations in each sector. It may be argued that based on the data presented here that particular tests should not be firmly recommended or counter-recommended for use in monitoring programmes in any particular sector. It may be wiser, given the limited scale of sampling presented here, to use all IWUAB-recommended tests for an initial year or two of monitoring, then to consider reducing the number of tests based on data from that site or region.

2.3.6.2 *Definitive-Effluent toxicity as EC_{50}*

The instream data were collected to assess the effect of effluent discharge on the resource. That is to say, if the sampling locations are chosen to include only effluent discharge and no other impacts between the upstream and downstream sites, changes in the results from the instream samples should reflect the toxicity of the effluent as moderated by dilution in the receiving water body. Effluent toxicities as effect concentrations from assessed sectors in the various definitive tests are presented in Figure 6. One immediate outcome of the use of definitive tests to assess toxicity that is apparent in Figure 6 is the relatively limited data set compared to the instream samples. The reason for this is the frequent failure of the test to return a valid EC_{50} score. This may happen for two reasons. Firstly, if the undiluted effluent is unable to have an impact on 50% of the test taxa in any particular test, then the effluent is insufficiently toxic for a valid EC_{50} score to be calculated. In this case, it may be possible to derive a different measure of toxicity, for example EC_{20} or EC_5. Secondly, if the effluent is extremely toxic, the dilution series selected for the analysis may be inadequate to produce results that allow the derivation of EC_{50}. This can be addressed by changing the dilution series to produce appropriate results, and repeating the analysis.

Both high and low effluent toxicities resulted in a failure to produce valid EC_{50} data for comparison with other tests. This happened in tests using samples from all sectors. In Figure 6, this outcome can be seen where data are missing, or where there were insufficient data to calculate a standard error term. For example, tests using bacteria on industrial effluent returned a mean EC_{50} of 85%, which is just toxic enough to produce an EC_{50}, and if the effluent were slightly less toxic, no derivation of EC_{50} would be possible. On the other hand, results from tests using fish on municipal effluent returned an EC_{50} of 6%. This indicates that the effluent is extremely toxic, and that should it be found to be slightly more toxic, derivation of a valid EC_{50} score would require repetition of the test with a modified dilution series. This would presuppose that sufficient effluent and materials were available and that the test could be repeated within a time frame that enabled appropriate sample storage.

Given that definitive tests may not return results, it is important to understand what proportion of tests did return results before the results presented in Figure 6 can be properly considered. Of the definitive tests conducted, only 17% returned EC_{50} data. Of those that did not return valid EC_{50} scores, 11% had toxicity that was too high to derive an EC_{50} given the dilution series used, and nearly all the remainder were from tests where the toxicity of the sample was too low to derive an EC_{50}. Of the data that were too toxic, 73% were from the municipal sector. Of the data that were insufficiently toxic, 42% were from mining samples, 32% were from industrial samples, and 25% were from the municipal sector. These figures are slightly skewed by the more frequent rate of sample collection in the municipal sector. Of the samples tested from the industrial sector, 71% had limited toxicity, and 5% had high toxicity. From the mining sector, 79% of samples had limited toxicity, and 3% had high toxicity. Finally, of the municipal samples, 15% have high toxicity and 37% percent have low toxicity. These data indicate the generally more toxic nature of effluent from the municipal sector. They also suggest that of the effluents assessed, mining is marginally less likely to be toxic.

Given that the majority of samples did not return valid EC_{50} scores, it must be emphasized that the data presented in Figure 6 represent 17% of tests, and are from samples where toxicity was both high enough to return a result, yet low enough that the test did not require repeating using a modified dilution series. The data in Figure 6 cannot therefore be taken as representative of all samples collected. In addition, given the lack of effluent in the agricultural sector and the fact that definitive tests were only undertaken on effluent samples, these results are not representative of all sectors surveyed.

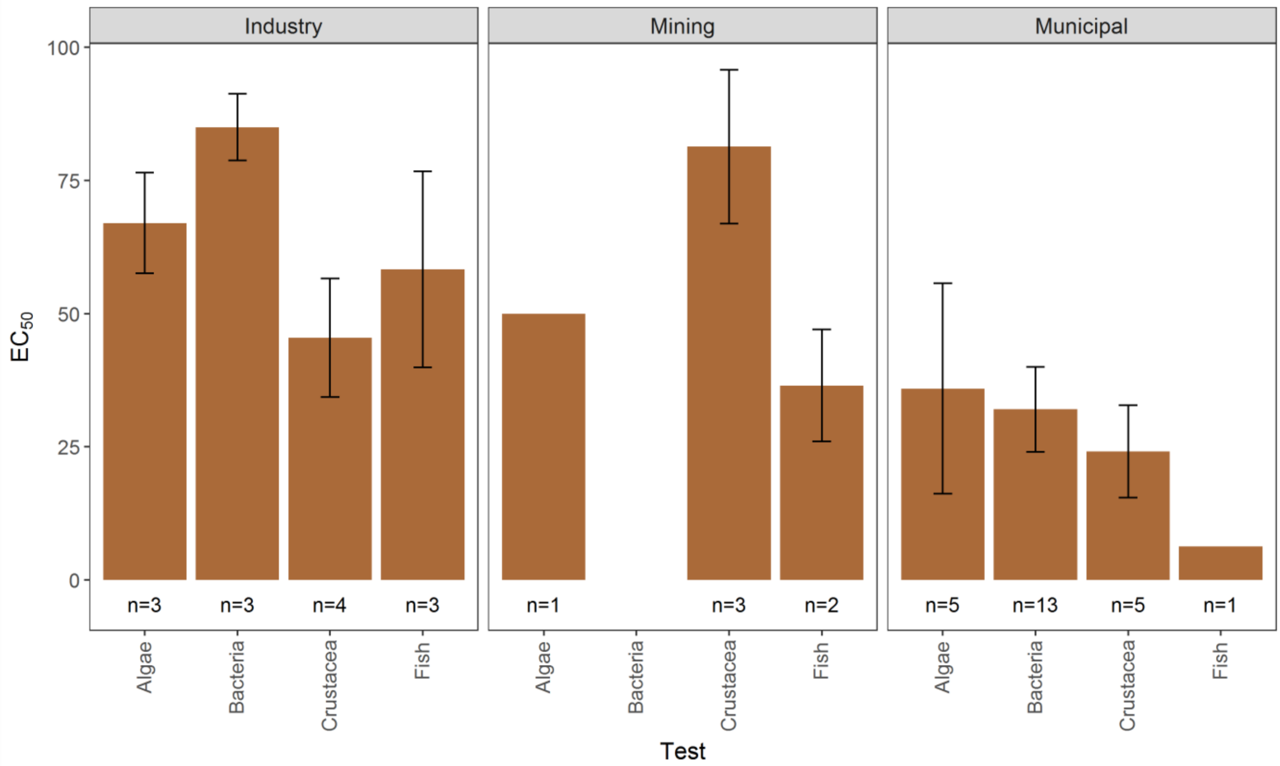

Figure 6: The results of individual toxicity tests by sector in effluent samples. No effluent was collected from agricultural sites. The results show the EC_{50} in percentage effluent for definitive tests. Plots show means ± standard error.

When valid EC_{50} results were produced, municipal effluent proved by some distance the most toxic effluent. The mean EC_{50} for this effluent ranged from 6% to 36%, meaning that a considerable level of dilution would be required to minimise impact on biota in the receiving water body (the sampling sites employed in this survey were in relatively larger water bodies). Effluent hazard classes from this sector ranged from class one to five, with hazard classes two (slight acute hazard) and five (very high acute hazard) being the most frequent, followed by class four (high acute hazard). The frequently toxic nature of municipal effluent affected all test organisms. The level of toxicity apparent in Figure 2 and Figure 6 has an impact on receiving water body toxicity at times, and effluent from this sector will need to be carefully managed.

Industrial effluent seemed, based on mean EC_{50} scores, to have a greater impact on crustacean and fish rather than on algae or bacteria. As regards crustaceans, this conclusion is borne out by the results shown in Figure 5, though less so for fish. Results from algal and bacterial tests are marginally toxic enough to derive a valid EC_{50}, and, as noted above, 71% of samples from this sector were insufficiently toxic for a valid EC_{50} to be derived. The generally less toxic nature of this effluent was confirmed by hazard classes that ranged from two to four, with most in hazard classes two (slight acute hazard) and three (moderate acute hazard).

Of the various effluents assessed, mining effluent had intermediate toxicity. Algal, crustacean and fish tests returned valid EC_{50} results, with crustacean tests returning marginally more valid scores than fish. Only one valid EC_{50} from algae was collected. Bacteria returned no valid EC_{50} scores, which was not surprising given the stimulation of bioluminescence apparent in Figure 5. The less toxic nature of this effluent is supported by the data presented in Figure 5, and the range of hazard classes for this effluent ranging from two to four, with nearly all being in hazard class two (slight acute hazard) or three (moderate acute hazard).

It must again be noted that data not representative of all samples from the various sectors, as samples where EC_{50} could not be derived owing to limited or excessive toxicity are excluded from this analysis of EC_{50} data.

2.3.7 Differences between catchments in upstream results

To address Aim 5, upstream data from differing catchments were assessed for catchment-specific limits to be derived. The scope of the project did not include surveying catchments across the entire country to determine limits, so data-based toxicological limits for all catchments in South Africa could not be recommended during this process.

The catchments that were surveyed were mostly broadly spaced around Johannesburg, with two from the Eastern Cape. Most drain to the Vaal River, though there were sites in the drainage basins of the Olifants and Limpopo Rivers. The Eastern Cape rivers drained to the Great Kei and Buffalo catchments.

Upstream samples were used to assess the state of the resource at time of sampling. No overt differences between catchments could be detected. The majority of samples, regardless of catchment, that were collected were in hazard class two (slight acute hazard), with a significant number in hazard class three (moderate acute hazard). A small minority of samples were in hazard class one (no acute hazard) and even less were in hazard class four (high acute hazard). Variation within sites was relatively high though, with many sites shifting from hazard class two to hazard class three with time, and many sites experienced within-site shifts in upstream toxicity of two hazard class units.

Given that within-site variability in general matched or exceeded between-site or between-catchment variability, no straightforward recommendations regarding differing limits for various catchments can be proposed from the data collected here. Even comparisons of catchments between the sample sites in and around Gauteng with sites in the Eastern Cape revealed no clear differences. The same was found for comparisons of sites in rural and urban environs.

Interactions with stakeholders generated the observation that the blanket compliance criterion currently produced by the IWUAB toolkit that downstream toxicity should be maintained below hazard class three at all times would render the compliance criteria legally indefensible in light of observed simultaneous upstream hazard classes of three or occasionally four. For this reason, several stakeholders from the regulator expressed the opinion that in the light of these recommendations, toxicological tests recommended by the IWUAB toolkit could only be used in a screening role.

Given that simultaneous upstream data are collected, and given that the results indicate substantial within-site variability in toxicity, legally defensible compliance criteria should only be defined by comparing upstream and downstream samples. This comparison will clearly demonstrate the impact of the effluent and so will not be liable for potential legal challenges.

Generalized criteria for catchments might be generated based on results from the NTMP. However, the programme is in its early stages and data available may prove insufficient at this stage. Little data from the programme has been published at this point, and the detailed data that would be required for such a task have not been reported on (D. Odusanya pers. comm.). Data from this project that indicate high within-site variability strongly suggest that such limits might not ever be defensible. However, as more data becomes available, recommendations can be refined based on knowledge of a system.

2.4 DISCUSSION

A significant difference in effluent toxicity between sectors was found, although this did not translate into a statistically significant difference at $p<0.05$ between sectors in terms of their impact on the environment. This is likely a consequence of the effluent discharging into rivers of different sizes and flow rates, which will result in differing dilution rates of discharged effluent. Dilution of effluent is a major factor in modifying receiving water instream toxicity (Diamond and Daley, 2000). It will also be affected by the differing upstream river toxicities, which varied from hazard class one (no acute hazard) to four (high acute hazard), with the majority of samples in hazard class two (slight acute hazard). If river-specific variability (which will have a spatial and temporal component) has such an effect on effluent impact, it should receive some attention when recommendations for monitoring are generated.

A number of samples were actionable according to the IWUAB recommendations that the difference between upstream and downstream sample's toxicity as assessed using the hazard class of Persoone et al. (2003) should not exceed one hazard class, and that the downstream sample hazard class should never be three or greater. Actionable cases with respect to a large change in hazard class of the resource from upstream to downstream were relatively rare, and only three instances from two municipal sites were noted. In contrast, actionable cases where downstream hazard classes were three or more were relatively common (28% of downstream samples), and occurred in all sectors.

In 46% of sites where the downstream hazard class was three or greater, the upstream hazard class was also three or greater. As such there is limited evidence in many cases that the effluent increased resource toxicity to a notifiable level, as downstream toxicity can completely be attributed to existing upstream toxicity. Further collection of instream toxicological data would shed more light on expected resource toxicity. Where an increase in toxicity from upstream to downstream was found, 53% of cases increased from hazard class two to hazard class three, 18% increased from hazard class one to hazard class two, and none increased from hazard class three to hazard class four. Interestingly, the number of upstream sites with a hazard class of three or more was greater than the number downstream, suggesting that point or distributed discharge of effluent might improve water quality in some cases.

The relatively high number of actionable cases where upstream hazard class two is exceeded (31% of upstream samples) indicates a widespread level of river toxicity that was not anticipated at the start of this study. This level of toxicity is present in the various receiving bodies before any effluent discharge (or other impact owing to water users) that was assessed in this survey. All sectors have at least one upstream sample in hazard class three (or more), regardless of the location of the sample site. Finally, no site is consistently toxic at the upstream sample point. Hazard class three indicates that the sample from the site killed or impacted at least 50%, but not 100%, of at least one of the test organisms. When compared with NTMP proposed guidelines for instream toxicity (DWAF 2005), this would result in every river that was sampled being classified as Poor at least some of the time.

The number of upstream sites where hazard class three is reached or exceeded invalidates the IWUAB recommendation that downstream hazard classes should remain below hazard class three. As it stands, the responsibility for ensuring that downstream toxicity is limited would fall on the water licence holder, regardless of the toxicity of water at an upstream site. This IWUAB recommendation will need to be reconsidered in such a way that all water use license conditions take account of the results from the upstream site. Given that no clear differences between sites were found to support the application of a generic compliance recommendation based on the catchment or other criteria, and given with within-site variability may be significant, it is recommended that criteria be phrased in terms of comparisons between upstream and downstream samples. Alternately, IWUAB licensing recommendations may be reviewed by an appropriate specialist once evidence is available.

The results presented here show multi-test toxicity in response to sectoral effluents, with all effluents returning varying test responsiveness. This validates the use of a battery of tests to assess the toxicity risk of particular effluent. Use of multiple tests is common in Whole Effluent Toxicity testing (Tonkes and Baltus, 1997), and was included in the methods proposed for use of DEEEP in effluent toxicity testing in South Africa (Slabbert, 2004, Pearson et al., 2015). The results presented here support continued use of this approach. The sensitivity of the various tests in response to various sectoral effluents is analysed and discussed in 2.3.6.1, and recommendations made as regards which tests are more sensitive in particular effluents. It may be possible to reduce the number of tests (as the IWUAB toolkit does in not recommending fish tests in the municipal sector), but use of multiple tests in each sector should continue.

Use of the hazard class system as advocated by the IWUAB has some advantages. Classes are easy to understand and may appeal to water managers, but they also simplify data and in the process, lose information. The class system devised by Persoone et al. (2003) is easily interpreted as all classes have clear meaning in terms of toxicological impact on test organisms (see Table 2). The class system builds on approaches used for WET testing internationally and was proposed for incorporation into DEEEP (DWAF 2003). The use of screening test results in the classification system simplifies the testing regime and therefore the costs of sampling. As noted above, the failure of the majority of data to return valid EC_x score in many cases would limit a classification system that relies on these data. The Persoone et al. (2003) paper has been cited 160 times (http:/www.scopus.com, accessed 9 April 2018), suggesting that the method and classification has widespread recognition, although not all citations used the classification system itself. The NTMP also use the Persoone (2003) classification (D. Odusanya pers. comm.) which indicates some acceptability within DWS.

A potential drawback of the method may arise when results from one site or occasion are close to a class boundary. For example, if an upstream site returned a percentage effect of 48% in the worst affected test taxon, this would lead to a hazard class of two. If a sample from a matching downstream site returned a percentage effect of 52% in the worst affected test taxon, this would result in a hazard class three score, which is the maximal change of hazard class from upstream to downstream following IWUAB recommendations. On the other hand, if an upstream site returned a percentage effect of 52% in one test taxon, and the matching downstream site returned a percentage effect of 95% in all test taxa, this would not result in a hazard class change. At an extreme, if an upstream site returned a percentage effect of 48% in one taxon, and a downstream site had a percentage effect of 100% in one taxon, this would result in an actionable change in hazard class of two units. In contrast, if the same upstream site had a downstream site where all test taxa had a percentage effect of 98%, the change in hazard classes would be one unit and therefore permissible. What these examples illustrate is not a particular characteristic of the Persoone et al. (2003) classification, but of classification systems in general. As is illustrated above, shifts over boundaries can occur as a result of small changes, and large changes may result in no change in classification.

Persoone et al. (2003), in testing the multi-test classification system they proposed, made extensive use of class weight scores to assess how much importance to attach to hazard class scores they found. Class weight scores formed no part of IWUAB toolkit license recommendations, and so were largely omitted from analysis in this report. In addition, an analysis of variance during the current research found no significant change in class weight scores in effluent between sectors, though the results suggest that a small increase in sampling effort might have given rise to changes that were statistically significant at the widely accepted 5% level. Given that class weight scores indicate how broadly the impact of toxicity may affect receiving water ecosystems, this may need reconsideration as these data could plausibly be used to support hazard class score data in license recommendations.

Variability in results was significant, which was not surprising considering effluent from various sites and times was used, and water bodies are not static. The relatively small scale of the project meant that sampling remained constrained by cost, and the scale of the project meant that many conclusions as to trends in the data would not be backed by clear statistical significance. This generality extends to several indications of trends identified in the graphic analysis above. For example, several trends identified in the results are not statistically well supported by data as indicated by the size of the standard error bars, and must therefore be considered tentative.

A linked issue is that of sample sites' representativity of their sector. With only five sites per sector, no set of data collected here can be taken as truly representative of that sector throughout South Africa. Selection of sites was further constrained by difficulty in forming partnerships with stakeholders which limited the types of sites available for selection. As an example, acid mine drainage is a major threat to water quality in South Africa (CSIR 2010, DWA 2011, Expert Team of the Inter-Ministerial Committee under the coordination of the Council for Geoscience 2010). This study used five sample sites from the mining sector, including at least one gold mine, and gold mines have been associated with acid mine drainage. However, no acidic or highly saline effluent characteristic of acid mine drainage was collected during sampling, and the results cannot therefore be taken to represent all mining impacts on water resources, including the receipt of treated or untreated acid mine drainage. Likewise the impact of coal mining effluent, copper mining effluent, etc. was not assessed. The results of the project must be considered in the light of the project goals. They are not a full survey of a particular impact, but rather a range of conditions to assess the application of the IWUAB toolkit in drawing up water use licenses.

Variation in instream results is largely a function of resource toxicity upstream. The downstream toxicity reflects the upstream toxicity to a large extent, and only a few examples found a notable impact of effluent discharge having a clear effect on downstream receiving water toxicity. The worst examples were found in the municipal sector where the effluent hazard class was five (i.e. where the effluent killed or inhibited all test organisms). However, there are earlier examples from this site where class five effluent had no detectable effect on instream toxicity. In one notable and extreme example, also in the municipal sector, receiving water at hazard class one received class five effluent, and no change in hazard class at the downstream site was found.

Despite variability in the toxicity of the receiving water body, it is clear from the results reported here that of the sectors receiving effluent, the municipal sector produced the most toxic effluent and had the most toxic downstream conditions. Industrial effluent was toxic, though less so than municipal effluent, and had no detectable impact on downstream resource quality. Mining effluent was often less toxic than or as toxic as the receiving water. No effluent was available at agricultural sites, and there was no detectable change between upstream and downstream sites in this sector.

2.5 CONCLUSIONS

Data reveal effluent from the municipal sector to be the most toxic, followed by industrial effluent. Mining effluent was not noticeably more toxic that the receiving water body, and no effluent could be collected from the agricultural sector. Receiving water bodies were least toxic in the municipal sector, with fewer clear differences between the remaining sectors. Receiving water bodies were relatively frequently found to be at hazard class three and occasionally four. This is worrying for the management of rivers is they are significantly toxic before discharge of effluent. The cause of this is not known. However, it's occurrence negates the possibility of using one of the IWUAB toolkit recommendations for water use licenses, namely that downstream hazard classes need to be kept above three at all times (unless this recommendation is adjusted when issuing or renewing licenses based on data from the site, or rivers are managed to bring toxicity to an acceptable level). The sensitivity of different tests in the various sectors varied, and the number of tests in each sector could potentially be reduced, based on the results presented here, or the first two years of data collected while monitoring a particular site. The use of screening tests as recommended by the IWUAB toolkit should continue, as definitive tests would require greater sampling and analytic effort to return results.

CHAPTER 3: IWUAB TOOLKIT REVIEW

3.1 INTRODUCTION AND OUTLINE OF GOALS

As a part of the pilot testing of the IWUAB toolkit, we assessed the operation of the IWUAB toolkit in the four sectors (agriculture, mining, industry and municipal) that it supported when this project commenced.

A guideline to the application of the toolkit was presented in Pearson et al. (2015), along with background to kit and design rationale. The toolkit design process incorporated input from government, industrial and consultant representatives regarding difficulties in applying bioassays in the water use authorisation process. It reviewed laws, guidelines and practices for water quality management and bioassay application in South Africa and internationally. Information on monitoring requirements and user experience with application of bioassays in water quality management led to the production of a toolkit to facilitate application of bioassays in water use authorisations.

The IWUAB toolkit currently can be used for input on appropriate bioassays in the freshwater environment in the agricultural, mining, municipal, and industrial sectors. The toolkit is envisaged to be expanded to include methods for estuarine and marine environment, to incorporate tests for sediment as well aquatic samples, and to incorporate methods appropriate to other sectors (Pearson et al., 2015). As such, the IWUAB toolkit can evolve with user and monitoring requirements.

The IWUAB toolkit, which was applied in the pilot test process described here, is a series of forms coded in Microsoft Excel. It was produced by Oliver Malete, Bridget Shaddock and Hesmarie Pearson under the auspices of WRC project K8/1070. The toolkit guides users through a series of questions related to sectorial affinity and water use, output requirements, description of effluent and sample type, previous monitoring results, catchment and river information, and potential for bioassay interference, to produce water license or monitoring recommendations. It also contains details on appropriate bioassays, their applicability, specificity and interferences, along with information on test taxa, test endpoint, etc., standards and references for tests using bacteria, algae, invertebrates, vertebrates and plants.

This section includes observations on the application of the toolkit to produce recommendations for conditions regarding bioassay application in water use authorisation in four applicable sectors. It largely comprises recommendations for the user interface and application of the toolkit in water quality management. This section does not include a consideration of success in application of the toolkit in water quality management, or theoretic qualities of the monitoring programmes that are recommended as these largely follow standard practice, but rather presents points related to application of the Excel-based interface in deriving bioassay monitoring requirements and applying these recommendations in practice. Observations will be grouped into major issues with the use of the toolkit and its recommendations, as well as a number of minor issues related to user interface functionality and ease or use. The majority of the major points relate to recommendations produced by the kit in the light of toolkit input and their potential for direct application without further refinement. Minor points are most commonly related to user interface design and ease of use.

This chapter has been produced to support on-going revision and evolution of IWUAB toolkit.

3.2 IWUAB TOOLKIT AND ITS POTENTIAL FOR IMPLEMENTATION OF TOXICOLOGICAL TESTING

This section contains observations that were made during the course of the project and during interactions with stakeholders from DWS, toxicologists, consultants, and project partners. Those presented in this section relate to broader issues related to practical implementation of IWUAB toolkit recommendations and the adoption of the toolkit as a tool in the water use authorisation process. Points related to the implementation of the toolkit's user interface are presented in Section 3.3 below.

3.2.1 IWUAB toolkit hazard classification

Aquatic toxicity testing is essential for determining the effects of a chemical substance to aquatic species and makes it possible to apply aquatic toxicity classification criteria to an aquatic ecosystem (i.e. both the biota and water resource). An acute (short-term) toxicity is expressed as the median lethal or effect concentration (LC_{50} or EC_{50}), which is the concentration that kills 50% or adversely affects 50% of the exposed population of test organisms after a continuous fixed period of exposure. Similarly, a chronic (long-term) toxicity is expressed as No Effect Concentration (NEC), which is the estimated concentration that marks the limit below which no statistically significant effect on the exposed population of test organisms compared to the controls is found. Traditionally, toxicity data are essential for determining the environmental hazard classification of a chemical substance, including wastewater and even suspected natural surface water. Lower LC_{50}, EC_{50} or NEC indicates higher toxicity, while higher LC_{50}, EC_{50} or NEC indicates lower toxicity.

Harmonisation of classification system for acute toxicity is necessary to establish a common risk that a chemical could pose to human and ecological health. Such a system would provide a common and coherent basis for chemical hazard classification and communication from which the appropriate considerations for environmental protection can be made. One approach of environmental risk assessment using aquatic toxicity data is by dividing the obtained LC_{50}, EC_{50} or NEC with various assessment factors (factors of 10) to calculate Predicted No Effect concentrations (PNEC) for the aquatic environment. The PNEC is the concentration of a substance or effluent in an environment below which adverse effects will most likely not occur during short-term or long-term exposure. The PNEC is then compared to Predicted Environmental Concentration (PEC) to determine if risk is controlled or not (Calow, 1998; Chapman, 2007).

Another approach of using aquatic toxicity data for environmental risk assessment is by applying the Species Sensitivity Distribution (SSD) technique. Application of SSD in ecological risk assessment involves combining data from single-species toxicity tests to predict concentrations affecting only a certain percentage of species in a community (Newman et al., 2000). For instance, the LC_{50} or NEC values from single-species toxicity data for many species are separately fitted to a distribution such as the lognormal or log-logistic. A hazardous concentration (HCp) at which a certain percentage (p) of all species is assumed to be affected, is then identified from the resulting distribution of species sensitivities. The most conservative form of this approach uses the lower 95 % tolerance limit of the estimated percentage to ensure that the specified level of protection is achieved (Newman et al., 2000).

The SSD and PNEC approaches can be used to protect aquatic ecosystems from risk of contamination as they are based on sensitivity of organism to toxic substances. However, Persoone et al. (2003) proposed another system of risk assessment that categorises the toxicity of natural waters as well as wastewater into different toxicity classes, whereby the assessment system of natural waters is referred to as the hazard classification system and that of wastewater the toxicity classification system. Although Persoone et al. (2003) focused on classification systems for short-term exposures, long-term classification systems can also be determined using the same principle.

The acute toxicity class determination involves a two-step process of exposing a battery of test organisms to non-diluted wastewater samples, then to a dilution series of the samples that resulted in more than 50% effect, in a short-term exposure. The effect results obtained with each test are transformed into toxicity units (TU). Based on the highest number of TU found in one of the test battery, the samples are classified into acute toxicity classes. Thereafter, to indicate the quantitative importance (i.e. weight) of the toxicity in each class, weight scores are calculated (Persoone et al., 2003).

The acute hazard classification of Persoone et al. (2003) is comparable to the Acute Toxic Unit (TUa) developed by the Netherlands (DWAF 2003). The TUa is derived from calculation in an acute toxicity test, whereby TUa = 100/LC_{50}, and classified as follows: <1 TUa = not acutely toxic; 1-2 TUa = negligibly acutely toxic; 2-10 TUa = mildly acutely toxic; 10-100 TUa = acutely toxic; >100 TUa = highly acutely toxic.

South Africa adopted the Dutch TEM approach in the Direct Estimation of Ecological Effect Potential (DEEEP) for ecological hazard assessment for wastewater, recognising ecological hazards may be different in different countries (DWAF 2003). In this study, both the hazard classification system for natural waters and toxicity classification system for wastewaters were used.

The Persoone et al. (2003) hazard class system is capable of generating results using screening tests alone, without a definitive result. This reduces the costs and effort attached to testing, and so will reduce the burden of toxicological testing for end users.

3.2.2 Toxicity guidelines

Guidelines produced by the IWUAB toolkit that state that the downstream hazard class should be maintained at less than hazard class three (moderate acute hazard) have been found to be inadequate during the course of the project. Data collected during the course of the research indicate that hazard class three occurs regularly at downstream sites, and that the primary reason for this is that the upstream hazard class is three, or possibly two or four. Assessment of the data revealed that the contribution of effluent toxicity to downstream toxicity was often low or undetectable. Given high upstream toxicities and limited demonstrated effluent impact, it is not tenable to place the responsibility for maintaining downstream toxicity levels at an acceptable level on the water user alone without reference to upstream toxicity.

It is important to note at this point the IWUAB guidelines are intended to act as a guide to a specialist setting conditions for water use licenses, and are not proposed as absolute limits (B. Shaddock pers. comm.). Given an understanding of the toxicity in the system in question, appropriate adjustment could bring these guidelines in tune with site-specific requirements.

Input from DWS staff at a public meeting held on 3 November 2017 indicated that the IWUAB recommendation that downstream toxicity should be held at less than hazard class three at all times would easily be challenged on a legal basis. This suggests that this recommendation is not legally defensible and so not appropriate for compliance monitoring.

Given that contemporaneous data on upstream toxicities are collected, it is therefore appropriate to assess downstream toxicities in light of a more site- and time-specific measure. The IWUAB recommendation that toxicity should not increase by more than one hazard class unit from upstream to downstream of effluent input is a good and clear example of a case where a direct comparative measure of toxicity at an upstream site before effluent impact is available. Other site-specific information like RQOs, or NFEPA, PES or REC class might also be useful. Comparison of river NFEPA class and downstream toxicity revealed that NFEPA class was of limited use as a predictor of downstream toxicity. As an example, upstream toxicities in municipal samples of hazard class one (no acute hazard) were collected from rivers classed as NFEPA class D and E/F.

On the other hand, upstream toxicities of hazard class three and even four (moderate and high acute hazard) were found in rivers classified as NFEPA class C, which was the highest NFEPA class in rivers assessed as part of this project. As the NFEPA classifications use a range of non-toxicological data (Nel et al., 2011b), this lack of correlation is not surprising. PES and REC classifications will also not closely reflect toxicological stress. RQOs that specifically mention toxicity might be useful however.

Use of upstream, un-impacted toxicity as a comparative measure to assess the toxicological impact of effluent on the resource is simple, clear and unambiguous. However, this is an approach that will not always be possible. Many of South Africa's receiving water bodies are ephemeral or non-perennial, and upstream flow is not always present (this occurred during the current research programme). In such a case, the upstream toxicity is not available for comparison, and in nearly all cases, downstream water is comprised of effluent alone and should therefore have the same or similar toxicity as the effluent. When this happened during the research, downstream toxicity was the same as effluent toxicity. In such a case, no receiving water is available to dilute or moderate the impact of the effluent. In the research undertaken here, a case was found where a river remained at hazard class one (no acute hazard) despite receiving effluent of hazard class five (very high acute hazard). This illustrates the capacity of the receiving water to buffer the impacts of the effluent. This will not occur when no upstream flow is available to dilute or buffer the effluent is present. RQOs might potentially provide a guideline where upstream flow is absent. Another possibility would be to use the PES/REC class of the river for the test taxon being assessed (where that is possible). Simple toxicological restrictions on released effluent would also be possible.

As a result of the above, monitoring guidelines determined by the IWUAB toolkit will need to be refined and clarified. The relatively high upstream toxicities and their effect in driving high downstream toxicities negates the appropriateness of application of a blanket rule regarding downstream toxicity levels as is currently present in IWUAB recommendations that downstream toxicity be maintained below hazard class three. Upstream toxicity also supports the IWUAB recommendations that downstream toxicities be assessed in light of upstream toxicity. However, while this is desirable, this assumes that upstream data are available. In the great majority of cases collected during this project, upstream data were available. However, there were cases where upstream flow was absent. Given recent droughts in South Africa (e.g. see Baudoin et al., 2017), this will reoccur (Davis-Reddy and Vincent, 2017), and the IWUAB toolkit will need to be able to make valid recommendations when it does. One potential route could be to assess downstream toxicity in the light of RQOs for the catchment. Another potential approach would be to compare downstream toxicity to that at a reference site in a similar, un-impacted site where flow is present (though in a drought, locating a comparative reference site may be a challenge). In considering an appropriate level of toxicity, one will need to consider that where upstream toxicities are not known, when this is a result of an absence of upstream water, downstream receiving water is likely to be comprised of effluent alone and so will reflect effluent toxicity levels. As more data on instream toxicity become available, setting of realistic instream goals will be easier.

3.2.3 Monitoring point selection and sampling strategy

IWUAB toolkit output should have more specifics on how far upstream or downstream of effluent release points receiving water samples sites may be located (Pearson et al. (2015) in the toolkit guidelines state a maximum of 5 km). In addition, there is no specific mention made of site selection in light of competing impacts in IWUAB toolkit output, although the guideline document has some site selection recommendations given competing impacts, these relate to point-source impacts and would not easily be adapted to account for difficulties in defensible site selection given the more diffuse impacts associated with the agricultural sector. Additional inputs complicating sample site selection have been relatively common in our experience. While offering specific recommendations to fit all possible cases is too much to include in a toolkit that advises on application of bioassays, some short but clear indication of what is required of a sample site is recommended.

For example, on agricultural properties with diffuse effluent release the distance between the upstream site and the downstream one may be large, as this is defined by the size of the property and not by the location of the effluent release point. With a large area, the chances of competing or interfering impacts will increase, and these may complicate monitoring. An example can be found at a farm(s) under irrigation at the confluence of the Rietspruit and Natalspruit just south of Katlehong in Gauteng. The farm or farms (landholder details are not known) is 3×1.5 km, and lies next to the Rietspruit, and approximately half way along the riverside the Natalspruit joins the Rietspruit. The water from the Natalspruit may have its own impacts (the 83 $Ml.d^{-1}$ Vlakplaats WWTW lies about 8 km upstream) and any sampling and analytic scheme will need to be structured around potential agricultural impacts owing to farm operation and other impacts that are related to Natalspruit water quality.

The above scenario considers one case where simple sampling recommendations will not deal with all cases that might be encountered. Another potential issue that was encountered is where the effluent represents several independent waste streams from independent sources. An example of this was found in an industrial park where several industries are present. Several stakeholders also raised this point at public meetings. At least two of the industries at the site produce effluent for discharge to river. All effluent leaves the park via a drainage canal, where it is mixed with any other effluent that might be present, and flows to a nearby river. If a different water use authorisation is held by each industry, then monitoring each to assess the impact of that industry alone will not be easy.

In the report that outlines how the IWUAB toolkit should be used, Pearson et al. (2015) note several points regarding site selection. They indicate that sampling points should be within 5 km of the potential contaminant source, and that no other potential contaminant sources should be present inside the monitored stretch. They also note that where such cases are present, monitoring could potentially be rationalized such that sites between impacts could produce data to be used for the monitoring both impacts. One impact's upstream site could then act as the downstream impact for another impact, and so on. Sampling in this way would require planning and coordination both between water users and during the authorisation process. As the above examples indicate, simple solutions may not be easy to come by, and sampling planning may affect users' sample effort and expenditure.

Final monitoring recommendations indicate that agricultural bioassays only to be undertaken "before and after seasonal events (e.g. spraying)". The nature of appropriate seasonal events that might trigger monitoring needs to be more clearly defined especially where activity is, for example, livestock farming. Alternatively, given the number of potential sampling scenarios is large, review of sampling recommendations by professional scientist is required.

3.2.4 Lotic and lentic water

The sampling recommendations in IWUAB toolkit output relate entirely to flowing water systems, with no consideration of standing water. Standing water might occur as a result of impoundment or simply reduced flow resulting in parts of a river where standing water is present. The phrasing of sampling recommendations in term of upstream and downstream do not account for potential impacts on standing water. An example of such could be agricultural land adjacent to dams. As implementation of the IWUAB toolkit to support toxicological testing is a priority, we do not propose that inclusion of sampling and monitoring recommendations adjacent to standing water is an immediate priority, but it should at a later stage be considered for incorporation during IWUAB toolkit revision and phrasing of IWUAB toolkit output should make this clear.

3.2.5 Use of input data in generating recommendations

The IWUAB toolkit collects data on river PES, but not, for example, RQOs, which, being managerial goals, are highly relevant. The collected PES data are not currently used in generating IWUAB toolkit recommendations (B Shaddock pers. comm.). However, given the evidence collected during this project of the degree of impact on receiving water, given managerial targets, and given the evidence collected here of current significant instream toxicity, it would be of considerable value to use these collected data to assess effluent impact on rivers of differing flow rates and ecological health. Once an assessment is complete, these data could be used in generating IWUAB recommendations. Given that RQOs reflect managerial goals as well as ecological health, they would be a valuable adjunct to PES. If toxicologically relevant RQOs are not available, data on the management class of the river could also be used in generating compliance criteria.

The IWUAB toolkit gathers data on receiving water flow rates in the wet and dry seasons, which would be of value in calculating seasonal dilution rates. However, no data are collected on effluent flow rates. Understanding effluent flow rates, together with river flow data will allow an assessment of effluent loading on the receiving water body. An understanding of effluent toxicity will inform assessment of hazard to the receiving water body. Finally, knowledge of river ecological health and managerial goals will inform generation of appropriate guidelines for the resource.

3.2.6 Endpoints of recommended tests

As currently implemented, the IWUAB toolkit uses the Persoone et al. (2003) hazard class system, where the hazard is assessed in terms of mortality, or acute hazard. Of the tests recommended by the IWUAB toolkit and applied during the research reported on here, two used mortality as an endpoint. Both used larger organisms with relatively long generation times (relative to testing time) and for these, mortality is an easily assessed and appropriate endpoint. The other two tests of the battery that were undertaken assess other endpoints in organisms with a short generation time relative to the test length. The algal test assessed population density at test end, and the bacterial test assessed bioluminescence at test end. Bioluminescence in *Vibrio fischeri* is triggered through quorum sensing, and is therefore a response to population size, though environmental conditions also affect bioluminescence and light production (Miyashiro and Ruby, 2012). As a result, both endpoints respond to population size, which, owing to the short generation time, can increase or decrease during the duration of the test. For example, output of individual tests showed that growth of algae was stimulated in municipal receiving water, though not in effluent. Assessment of direct test results reveals that algal growth was inhibited in levels of effluent as low as 6%. Bioluminescence in bacteria was stimulated in mining effluent and receiving water. Bacterial stimulation in mining effluent occurred at a range of effluent concentrations.

Because of the use of an acute hazard classification, stimulation effects do not play any role in hazard classification. Stimulation of growth of algae is a threat to water quality in South Africa, as eutrophication of water resources has been recognised as an important and growing threat to water quality in the country (CSIR 2010; DWA 2011; Harding, 2015). While not a toxicological hazard per se, the results do represent a response to effluent or receiving water body water, and therefore are indicative of likely ecological effects of effluent discharge. As the IWUAB is a toolkit to enable application of acute bioassays in water use authorisation, it is currently unlikely to be able to recommend use of data such as these. Nevertheless, these data represents a loss of information that could be useful in predicting the ecological hazard of waste discharge, and should form part of license review and renewal processes.

3.2.7 Use of effluent data

It is noteworthy that the effluent stream is sampled under the monitoring protocol recommended by the IWUAB toolkit, but the results of sampling the effluent are not used in any recommendations for inclusion in water use authorisations. DEEEP, a suite of tests for assessment of effluent and receiving water that reached pilot-scale implementation, never established limits to toxicity for effluent discharge or surface water toxicity (Chapman, 2011a). The approach that IWUAB toolkit uses is to assess toxicity in the light of resource toxicity changes without direct assessment of effluent toxicity, although toolkit output mandates an assessment of effluent toxicity. Data on effluent toxicity are collected but not used in guidelines generated by the toolkit. Given that these data are not recommended by the IWUAB toolkit for use in the water use authorisation process, an argument could be made that these data need not be collected except in response to an instream toxic response. However, these data monitor effluent toxicity and so are able to directly evaluate the likely response to this stressor, particularly given information on relative flow rates and effluent and resource toxicity. They are also important in assessing ongoing toxic loading of the water body in question, and therefore the potential cumulative impact of multiple stressors on the resource. They are also available to provide an ongoing record of toxic inputs to the receiving water, and to support revision of the toolkit and the monitoring conditions as part of an adaptive management programme. Finally, they could be directly incorporated into discharge licence requirements.

3.2.8 IWUAB toolkit sampling recommendations and ongoing monitoring

The data presented here may be used to argue for modification in the recommended monitoring programme generated by the IWUAB toolkit. Before continuing, it is important to note that the sampling frequency recommended by the toolkit and adopted for this project is proposed as an initial sampling frequency that can be modified after data are available to support changed monitoring frequencies or triggers (Pearson et al., 2005). The data collected during the research highlighted two trends that may be used to support modified sampling regimes: the toxic, though variable, effluent in the municipal sector, and the lack of detectable impact in the agricultural sector.

Results from the municipal sector indicate that the effluent is often, but not always, highly toxic (57% of samples indicate high or very high acute hazard). Despite the high effluent toxicity, evidence of impact on the resource is far more limited to the extent that clear negative impact (taken as upstream-downstream hazard class difference of two) is rare. Given the extreme toxicity of effluent in this sector, one can motivate for a higher monitoring frequency in the absence of evidence of reduced toxicity. Decisions in this regard may be made by the water authority if presented with clear and compelling evidence.

The sampling scheme adopted during this research for the agricultural sector was more frequent than the IWUAB toolkit recommended for model agricultural sites. IWUAB output for the agricultural sector (crops, livestock) stated that sampling should occur "before and after seasonal events (e.g. spraying)". This seems to imply that sampling should take place at least twice a year (although the inclusion of spraying and nothing else may be taken to imply that, in the absence of spraying or other seasonal impacts that clearly may impact the resource, there need not be any sampling at all). The Pearson et al. (2015) IWUAB guideline is more specific, stating "for the first two years sites should be monitored a minimum of four times a year (quarterly). After this initial monitoring period, only if no toxicity has been observed, can the monitoring schedule be altered to twice a year (biannual)". They also note the importance of seasonal events. Monitoring recommendations differed for aquaculture and processing plants, neither of which were assessed here, and so are not considered.

The results from the agricultural sector found no impact of agriculture at sites that consisted of irrigated sites with one feedlot. At no site was the downstream site at a higher hazard class than at the upstream site, and on four occasions the hazard class improved from upstream to downstream. The remaining sites had no change in hazard class from upstream to downstream. The number of sites assessed here are too few to draw general conclusions about agricultural impacts, and certainly not to conclude that all feedlots pose no risk to surface water (e.g. see Jonker et al., 2009; Leet et al., 2012; He et al., 2016). Given though that four of the five sites assessed were under irrigated agriculture with various crops, it seems that in certain cases the impact of irrigated agriculture may be insignificant (but see, e.g., Mortensen et al., 2016; Merchán et al., 2018). It was not known whether crops at any of the sites were sprayed with pesticides or herbicide during the period of assessment. Likewise, although only one feedlot site was assessed, it appears that the toxicological impact of a feedlot on an adjacent river need not be significant. Given the lack of recorded impact, it will be necessary to clarify the recommendations generated by the IWUAB for the agricultural sector, to outline clearly when sampling is required and when it is not, and to refine sampling frequency and timing recommendations.

3.2.9 Cost

The cost of sampling, analysis and reporting may be a challenge to smaller operations, for example small and emerging farmers. Taking the costs of analysis and reporting that the project experienced, a hypothetical farming operation with upstream and downstream sampling, four times a year, using four screening tests would experience costs of approximately R32 000 per year. If sampling of effluent was added, costs would increase to R49 000 per year. Definitive tests, should they be required, would further increase the cost to R50 000 per year, or R74 000 if testing of effluent was undertaken. For comparison with these analytic costs, the cost of a full-time worker paid at the agricultural minimum wage for 1 March 2018 to 28 February 2019 is R47 630 (Department of Labour Sectoral Determination 13: Farm Worker Sector, http://www.labour.gov.za/DOL/legislation/sectoral-determinations/sectoral-determination-13-farm-worker-sector).

It should be noted that this approximate costing is based on a sampling frequency that collects four samples per year. The IWUAB recommendations indicate that sampling should be undertaken before and after "seasonal events (e.g. spraying)". As the recommendation only mentions spraying as an example of a seasonal event, one could potentially argue that in the absence of spraying no sampling would be necessary, or that simple seasonal monitoring should be implemented. As the IWUAB stands, this decision would rest with the officials producing the Water Use License. As a result, the costs of sampling would vary.

These costs of sampling could easily be managed by a larger operation with higher margins, but could be challenge to smaller operations. Smallholder farm development and support as a means of poverty alleviation in South Africa has been adopted under the New Growth Plan (ECD 2011; Thamaga-Chitja and Morojele, 2014), although it is recognised that the policy environment can pose a challenge. Adding a cost that is equivalent to at minimum two thirds of one worker's wages will pose a further hurdle to aspiring small farmers.

Potential areas for reducing costs could also involve cost sharing by neighbours, where two farms with diffuse inputs share the same stretch of a river. In such a situation, where the impacts of one farm would for the most part be indistinguishable from the other, combining the costs of sampling and analysis would reduce costs for the farmers in question. This scenario would rely on the two properties having relatively minor and comparable putative impacts. Where this is not the case then then sampling would need to be devised to best differentiate the impacts of the two farms.

The data collected during this project revealed no detectable impact from agricultural activity. At sample site 71057, the upstream and downstream sites were separated by 4.5 km. Samples collected here showed no change in toxicity from upstream to downstream (although no upstream data are available for December 2016 owing to a lack of water at the time of sampling). Satellite photography reveals that within this stretch between two accessible sample points, there appear to be several farms. If a farm is taken to be a clearly demarcated agricultural unit with associated buildings, there were 10 farms in this stretch, with the smaller ones being about 20 ha. Most of these showed clear signs of recent soil tillage on half of this area only. The primary crop grown in the stretch as seen during sampling trips was maize. Under these conditions, the cumulative impacts of an estimated 10 farms growing maize in an irrigation project on river toxicity was not detectable. By inference, the likely impact of each of the farms in this stretch would be undetectable.

As a result of the absence of a clear impact on riverine toxicity of agricultural activity, and given the potential for the cost implications of sampling to significantly impact small and emerging farmers, it is recommended that water authorisations in this sector take a particularly site-specific approach. It is clear that sampling would definitely be required when using biocides, and other higher-impact forms of agriculture and processing would also require monitoring. On the other hand, we demonstrate here the limited toxicological effect of some forms of agriculture. It may in these circumstances be necessary that the IWUAB toolkit recommendations remain general with the clear indication and understanding that these might be modified in the authorisation process in a site-specific way.

3.2.10 Data storage

The IWUAB toolkit has been proposed as a means by which data on licensing or authorisation with regard to toxicity testing requirements can be stored. Given the current format of the toolkit as an Excel application, this could only be undertaken by saving each spreadsheet once license conditions are generated under a unique and identifiable filename. This would generate a large number of individual files, each of which would contain the details of one application. Curating and managing the files would not be trivial task, and the likelihood of data loss is high. We recommend that data storage should be migrated to larger centralized database server or to the cloud. This would facilitate data management, backup, and review and analysis of the data, as all pertinent information would be available from one source to support this. As it is possible to use Excel as a front-end to a database server, the current Excel interface can be maintained, but a process added that on the conclusion of generation of output that all input data and monitoring conditions are passed for storage to a database server. This would maintain most of the current functionality without modification, and simply add a short step where data are transferred to a central server. An alternative would be to recode the entire interface to a dedicated application which could access data stored in a central location. The latter would allow far greater flexibility in design and implementation of the interface.

A centralized server for data storage could potentially be part of the DWS WMS database (Dr S Jooste, pers. comm.). Implementation of this option would require the support of DWS. Should this option be implemented, it would be of value to implement this as part of a system that stores all information related to water use licensing and license conditions.

3.2.11 Industrial effluent discharge to sewer

During the course of this project, we were surprised to find that most industries that we surveyed or approached as potential partners discharged any effluent they produced to sewer. Of the WWTW that our municipal partner operates, the proportion of influent treated that derives from industry ranges from 0-40% of total influent (other WWTW have higher industrial loads (Manickum and John, 2014). Our results do not indicate any clear link between WWTW effluent toxicity and the proportion of industrial influent treated (there was not a clear link between plant size and effluent toxicity either, although the largest plant produced the least toxic effluent). Under these circumstances, the responsibility for discharge of adequately treated industrial effluent falls on the WWTW operator rather than the industry itself (given that the industry will have to comply with by-laws and guidelines related to sewer disposal of effluent).

This highlights two obvious conclusions: a great deal of industrial effluent enters rivers after processing in WWTW rather than individually; and relatively little industrial effluent enters rivers directly. Industrial input by WWTW is not always removed by WWTW processes (e.g. Molokwane et al., 2008; Manickum and John, 2014; Agunbiade and Moodley, 2015) and the chemical constituents of influents will vary between plants and may modify the quality of WWTW effluent.

Given that much industrial effluent reaches surface water resource via WWTW rather than by direct emission to the resource, and that the IWUAB toolkit is intended to recommend an appropriate testing routine for industrial waste, the IWUAB toolkit should be re-assessed and potentially modified to account for industrial effluent discharged to sewer. This may involve recommendations for discharge permitting rather than looking at WWTW water use authorisation. One potential way this could take place would be to include criteria in the toolkit where one could select discharge to resource or to sewer. The IWUAB toolkit could then either continue with a licence application, or apply for discharge license to sewer.

3.2.12 IWUAB toolkit and implementation of toxicological assessment

In meetings throughout the project, several DWS staff have indicated that they see the IWUAB toolkit as of value in incorporating toxicological monitoring in South Africa, but propose that at least initially the toolkit should be used in a screening role and should not be used to define compliance criteria. In this light it is worth noting that the criteria proposed as draft toxicity criteria for the resource under NTMP in DWAF (2005) are far stricter than the ones proposed by the IWUAB toolkit. However, the IWUAB toolkit was designed for application in the water use authorisation process and this is the most appropriate place to pilot its implementation. It is also worth noting that the guidelines produced by the IWUAB toolkit act as recommendations to licensing, and are not a boilerplate standard. Licensing recommendations and review should be undertaken with input from a professional scientist (B. Shaddock pers. comm.).

It is also important to consider the focus of the IWUAB toolkit compared with that of the NTMP. The toolkit aims to facilitate implementation of toxicity monitoring of effluent in South Africa, and where it assesses resource toxicity, it is in the light of assessing effluent toxicity and its impact on the resource. The toolkit therefore acts as a source-directed control (SDC) in this context, although an assessment of resource toxicity is undertaken. The NTMP is directly concerned with resource toxicity, and does not of itself deal with effluent toxicity. The NTMP is a monitoring programme that forms part of resource-directed measures (RDM). The two are therefore different in focus and full congruity between recommended standards is not to be expected.

This project and Pearson et al. (2015) have engaged with DWS personnel regarding the IWUAB toolkit. In the latter case, it was with the aim of moving towards implementation of toxicity testing, a process that led to the production of the IWUAB toolkit. The current project has presented the kit to stakeholders and undertaken training on the toolkit. As a part of this process, we have received feedback on the kit in its current format. From feedback (see Appendix C) and pilot-testing of the toolkit it becomes apparent that changes will need to be made before the kit can be used in water use authorisation. This report provides feedback to support finalizing the IWUAB toolkit. It is nevertheless strongly recommended that finalization of the toolkit involve adequate input from appropriate DWS staff to enable both formal recognition of the value of the toolkit and its adoption as a tool that is applied by DWS staff. The final refinements to the toolkit to enable its adoption will need input from DWS to enable buy-in to the process and adoption of the toolkit. In particular, DWS input will be necessitated to finalize the compliance criteria proposed by the toolkit. One of the two suggested criteria were found to be legally indefensible during the course of this research. DWS input will ensure that finalized compliance criteria accord with other DWS programmes.

The IWUAB toolkit has been one more step in a long process in implementing toxicological monitoring of effluent in South Africa (see DWAF 2003; Slabbert, 2004; Chapman et al., 2011a, 2011b; Griffin et al., 2011; Slabbert and Murray, 2011; Pearson et al., 2015). The toolkit was produced as a way to assist regulators without specific toxicological skills in assessing and managing the effluent discharge process. This research process found that the tool had considerable value, although criteria produced by the toolkit need some revision. Once this is complete and the toolkit is adopted by the regulator, it will provide a method for operationalizing toxicological testing of effluent, and assessing its impact on the resource in South Africa.

3.2.13 Other

Shorter points that were noted during the project or raised at public and steering committee meetings that have not been addressed elsewhere in this document are compiled below.

It is important to note that the majority of the data that is gathered by the IWUAB toolkit is currently not used in generating monitoring recommendations. Its inclusion in the kit in the current implementation has been indicated to be for the purposes of gathering data to support further development of the IWUAB toolkit and implementation of sampling and analysis (B Shaddock pers. comm.). Currently, selection of monitoring programme and guideline production is guided by information on the sectoral affinity selected.

It was found the different tests undertaken during the course of the project responded differently to different effluents as well as sectoral receiving water samples. For example, in the mining samples assessed here, taken from mines in the gold/platinum sector, bacterial responses were stimulated. On the other hand, agricultural samples (taken from receiving water) showed a disproportionate inhibition in bacterial tests, when compared to other tests undertaken. Given the varying sectoral test responsivity, it is worth considering including weighting tests differently in different sectors.

IWUAB toolkit sampling recommendations should be flexible enough to fit in with other sampling programmes and so to facilitate the monitoring process.

All sampling and analytic processes recommended by the IWUAB toolkit need to be legally defensible or compliance with recommendations will not be legally enforceable. Legal input will be required to advise regarding the phrasing of recommendations to ensure that these are defensible.

The use of the IWUAB toolkit in generating authorisation recommendations and monitoring strategies will be greatly improved if results of monitoring are retained and stored by the regulator. This will provide a growing pool of sector-specific data that will enable regular and appropriate revision of the methods, tests and strategies adopted to support development of an effective monitoring strategy for each sector. There is no significant publically accessible pool of these data at the time of writing, and their generation and analysis will greatly facilitate development of these methods and improve water resource management with time.

DWS water management strategies relate recommendations regarding water quality to particular end-user requirements. Where the IWUAB toolkit refers to sectors relates to the anticipated effluent type and toxicity. In section C of the toolkit, users are asked to identify the most sensitive receptor in the resource (12.7). The following choices are presented: Animals; humans; and the environment. In its current form, recommendations vary by identified sector only and other inputs are intended to gather data for use in ongoing toolkit refinement (B Shaddock pers. comm.). As such, identification of the most sensitive receptor does not modify IWUAB output at all. The IWUAB toolkit sensitive receptors list does not concur with the end users selected for the water quality guidelines (for example see DWAF 1996).

3.3 USER INTERFACE AND TOOLKIT FUNCTIONALITY

This section consists primarily of observations on the user interface of the IWUAB toolkit. The points raised here are largely related to tuning the interface to facilitate production of a polished and functional product. Points related to data storage and the potential for centralized data storage were dealt with above.

- Choosing a "2 Water Use Main Sector" (Section A) does not cause any filtering of "6.2 Type of facility" (Section B1) which always lists facilities that seem to be mostly mining related.
- When testing water where effluent is explicitly non-point source in the agricultural sector, the final recommendations include sampling upstream and downstream in the receiving water body and another from the effluent. In this case, no effluent is present for sampling. The phrasing needs to reflect this.
- "Life stock" in Section A.2.1 should be livestock.
- Estimated average dry season flow in Section C 12.4 should be left-aligned.
- Drop or hide selections that are not fully implemented as yet in drop-down lists. Inclusion of menu items that are not fully implemented and supported clutters the user interface and may confuse or frustrate some users. Items should be retained where their function is for gathering data with the aim of refining of the toolkit, but where this is not the case, they should be removed or masked/hidden. Inclusion of hidden items will allow for collection of data on these without requiring user input (for example, a list where only item is currently present for selection).
- The Section C catchment identification subform/pop-up allows selection of the quaternary catchment to filter the list of rivers that can be selected, but at the moment is not able to filter to give primary drainage identification. In addition, the primary drainage list should have "Keiskamma" rather than "keiskamma".
- Mining as a water use sector in Section A 2 only gives gold, platinum, open cast coal, and copper mines as choices. There are no other options (and no choice of "other mine"). Note the Department of Energy reports that 51% of coal mined is underground (http://www.energy.gov.za/files/coal_frame.html) (though South Africa's coal is in unusually thick, shallow seams) and the impacts of this are largely known and similar to open cast mines. Underground coal should be added as a choice, or the selection should simply reflect coal mines. Other mines, for example chrome, should also be considered for availability.
- Section B.2 (ongoing compliance): beeb should be been.
- Bacterial test page: "Determination of the inhibitory effect of water samples on the light emmission of Vibrio fischeri": "Emmission" should be "emission".
- Section B1 point 6.3: Should runoff be considered separately from wastewater?
- Section D states "An 'aquatic sample type' has been automatically pre-selected". No preselect is in place, although the alternate selection ("Sediment") is not available.

- IWUAB recommendations state that "The Hazardous class should be maintained". This should be "Hazard class".
- Section C, catchment information pop-up: when a quaternary catchment is selected, this filters the list of river names to those in the catchment. Unnamed rivers are often listed as "Tributary", and, when more than one is present, it is not possible to distinguish between them.
- Section A: when a sector is selected, the sub-sectors available are filtered to match the selected sector. When industry is selected as the sector, the sub-sectors that are listed are textile, paper, power and petroleum. This is a very limited list, and in general covers sub-sectors that are established or potential polluters. Nevertheless, other sub-sectors need to be included, or alternately a catch-all category such as other needs to be added. Examples of sub-sectors that commonly emit effluent but are not included are breweries, and dairy or food processing.

3.4 DISCUSSION

The IWUAB toolkit provides a useful tool towards implementing toxicity testing of effluents given identified lack of toxicological capacity at the regulator (Pearson et al., 2015). The production of a toolkit that identifies potential tests has been undertaken before (Slabbert and Murray, 2011), but the IWUAB toolkit ties the output closer to what is required for monitoring, including recommending tests that are available at South African toxicological laboratories, sampling recommendations, and suggested compliance criteria. The interface is robust and it produces a clear outline of sampling and analytical requirements and suggested compliance criteria.

The IWUAB toolkit currently is valuable in suggesting bioassays, sampling programmes and compliance criteria, and in conjunction with accompanying documentation, provides considerable support for non-expert implementation of toxicological monitoring. Ongoing refinement is anticipated and desirable, but the kit as it stands fulfils most of the requirements for support of application of toxicological testing in many contexts. However, the IWUAB toolkit needs some refinement before it can be considered for adoption or widespread application in the water use authorisation process. This chapter of the report outlines areas where the kit will need modification, based on input from DWS staff, stakeholders, and observations throughout the pilot-testing stage. In general, the modifications are not large, but they will be needed to ensure that the output is legally defensible and suitable for adoption into a DWS monitoring programme.

Many relatively minor points raised above will not be specifically addressed here but should be considered during modification of the toolkit. This text will briefly consider the broader changes that are required to bring the kit to a point where adoption of the toolkit might be considered.

Perhaps the aspect of the kit that requires the most attention is the suggestions for compliance criteria that are produced by the toolkit. The toolkit needs to produce suggestions for criteria that are legally defensible, implementable, and compatible with extant DWS classification and monitoring programmes. This pilot test has identified one criterion as clearly not legally defensible. Suggested criteria in all cases assessed by the project team included a requirement that downstream toxicity should be maintained at below hazard class three at all times. The suggested criterion does not link downstream toxicity to upstream toxicity in this criterion, although another criterion, which states that the hazard class should not decrease by more than one hazard class unit from upstream to downstream, does. Sampling undertaken during this research found that upstream samples, prior to any impact owing to emitted effluent, were relatively commonly at hazard class three, and therefore that downstream samples at hazard class three might simply be a function of the condition of the resource.

Given variation in the resource, it will not be legally defensible to simply apply a blanket criterion to downstream sites. A simple blanket rule also ignores the existence of other managerial tools such as RQOs.

In contrast to the blanket criterion noted above, another criterion compares upstream and downstream toxicities to make compliance recommendations. This provides a far more defensible criterion. It is worth noting that to shift by two hazard classes can imply a large change in toxicity. For example, moving from hazard class three to hazard class five can imply a shift from 50% of one test taxon experiencing mortality to complete mortality in all test taxa. When acute hazard is lower, it could imply moving from no mortality at all in any taxa to at least 50-99% in one or more test taxon. If this criterion was stricter and a shift by one hazard class unit was actionable, then barely significant changes could trigger non-compliance.

IWUAB toolkit also contains suggestions for sampling requirements. The toolkit outputs on sampling are brief and will not cover all circumstances. Sampling strategies relating to conflicting impacts are not well considered. These are not likely to occur often when a point source of effluent is considered, but where potential effluent impact is diffuse, as in many agricultural cases, this must be carefully considered. In order that the outcomes are defensible, sampling will need to be done in such a way that the effects of different impacts can be distinguished, and this needs to be clear in IWUAB output. Another different case that is not considered is where effluent streams are mixed before reaching the receiving water, when different authorisations apply to effluent producers. If a single authorisation can be obtained for all effluent producers this will not be an issue.

All IWUAB outputs prescribe an effluent sample, and an upstream and downstream sample in the receiving water body. Compliance recommendations are entirely based on instream results, and do not directly use data from effluents at all. Given the ephemeral nature of many of South African water bodies, many upstream sites may have no flow or water that can be sampled. This removes a sample that is used to assess compliance at the site. The blanket rule referred to above assessed compliance using only a downstream sample. We have illustrated how this is not legally defensible in its current recommendations. However, when upstream flow is absent, effluent flow or downstream flow, which will consist primarily of undiluted effluent, is the only thing that compliance can be assessed on. While it will not be legally defensible to set criteria based on downstream flow when upstream toxicities may be high, in the absence of upstream flow, criteria can only be based on effluent or downstream sampling. In such cases, criteria should reflect management goals for the receiving water body.

The associated guideline for use of the IWUAB toolkit (Pearson et al., 2015) has more detail on sampling requirements than the output of the toolkit itself. We recommend that toolkit output should contain more sampling detail than it currently does, or should explicitly refer to the guideline for sampling requirements, particularly when sampling recommendations may need to consider site-specific conditions as above. Input from a professional scientist should be included in planning the sampling process.

It was found during the course of the research that the majority of identified industrial effluent was discharged to sewer and not to surface water. In cases industrial effluent formed a major part of the inflow to wastewater treatment works, though some processed no known industrial effluent at all. Depending on the type of effluent and its potential toxicity, this has major implications for operators of wastewater treatment works. There are also legal implications as the responsibility for discharge of treated effluent devolves from the industry to the wastewater treatment works' operator. Given that discharge of effluent to sewer will be governed by local by-laws or other regulations, it is not clear whether it is worth considering adopting the IWUAB toolkit at this point to cover effluent discharge to sewer. Some industrial effluent is discharged to surface water bodies though, and the IWUAB toolkit will need to retain the capacity to advise on testing of these discharges to surface water.

The IWUAB toolkit itself is robust and easy to use. The user interface is relatively straightforward, and the number of changes recommended above are few. Other recommended changes relate mainly to data storage for adaptive management and progressive modification of the toolkit. The IWUAB toolkit requests a range of data to generate its output, but uses very little of this. The data is currently retained to support an adaptive management process leading to refinement of the toolkit. In its current form, the toolkit is implemented as an Excel application, and a new file would be required for each use. This would not make the data easily accessible for toolkit refinement. It is recommended that data from the kit be stored in a central database, to enable assessment of trends in input data. Toolkit refinement would greatly be facilitated if this data were stored together with the results of tests as this would enable assessment of results in the light of input data, and would improve sampling and testing requirements. These changes would not be required for a pilot introduction of the toolkit but would greatly facilitate ongoing toolkit development. Steps have been taken in this regard, but issues of hosting and ongoing management have slowed progress (B. Shaddock).

As noted above, development of the toolkit and the pilot-test described here have consulted with a range of stakeholders, which have included appropriate DWS staff. However, development and testing have been somewhat isolated from the department. This report presents a range of changes that would be required for adoption of the IWUAB toolkit into the water use authorisation process. Many changes are recommended here. But in order to produce a tool that is of genuine value in the water use authorisation process, particularly if the toolkit is to lead to production of valid and legally defensible compliance criteria, it is essential that DWS and CMAs are consulted throughout the process of finalizing the toolkit prior to its adoption and use.

Interactions with the regulators will need to involve representatives of appropriate seniority and responsibility in order that final modifications best reflect the requirements of the regulator. During the process, it will also be necessary to determine the step required towards finalization of the toolkit to fit departmental requirements, its piloting, and its anticipated role within the authorisation process. Without this engagement, the likelihood of the IWUAB toolkit achieving widespread application in support of adopting appropriate routine toxicological testing in effluent control and environmental water quality management will be limited.

CHAPTER 4: CONCLUSIONS & RECOMMENDATIONS

4.1 CONCLUSIONS

4.1.1 Sectors, impacts, analyses and sampling.

Sampling schedules and recommended tests put forward by the IWUAB for different sectors were based on prior professional experience with the sector in question. Municipal effluent had a frequent testing schedule owing to known toxicities and the data presented here confirm the toxicity of municipal effluent. Sampling in the agricultural sector was more flexible and allowed for reducing testing to twice a year on a seasonal basis depending on identified impacts. The complete absence of any toxic impact reported on here from the agricultural sector suggests that these are adequate, though this will depend on site-specific understanding of farm practices and likely impacts. The absence of detected impacts, and the known cost of toxicological analysis, indicates that consideration needs to be given to when to implement toxicological monitoring in this sector. Nevertheless, the dataset presented here is small and does not cover all potential agricultural impacts, and the potential for monitoring should always be assessed in the licensing and licence review process.

The results reported on here found that municipal effluent was far more toxic than other effluents. This is worrying given other identified threats to water quality posed by municipal effluent (CSIR 2010; DWA 2011). The recommended sampling for municipal samples included three analyses using bacteria, algae and crustacea. Infrequent fish-based toxicological analysis was undertaken to assess the potential value of including this test in analyses of effluent from this sector. Fish responded less to municipal effluent than other tests applied to that sector, and the responses from fish tests of the resource indicate no detectable impact of effluent discharge. Use of fish tests on the municipal effluent would not change the classification of effluent toxicity, but may have indicated higher toxicity in the resource as fish were the most sensitive taxon in response to resource samples from the municipal sector. Based on the data presented here, fish tests add limited value to the assessment of the resource, but may be of value if valid direct comparisons of the resource in the municipal sector with other sectors (where the fish test was included) are to be made.

Samples from the industrial sector included the results of tests on effluent and the receiving resource associated with three wastewater treatment works receiving a large proportion of influent industrial waste. Despite this, the toxicities found in this sector were lower than those found in the municipal sector. An assessment of hazard class scores in the municipal sector found that high municipal toxicities derived from wastewater treatment works with minimal or no industrial input as well as one with a relatively high input. The impact of industrial influent on wastewater treatment works effluent toxicity was not simply predictable in terms of industrial influent load. Despite the use of wastewater treatment works with high industrial influent loads as indicators of potential industrial, the toxicities found in samples from the industrial sector were lower than those found in the municipal sector. Samples taken from industrial effluent alone (textile and dairy/beverage industries) could not be clearly distinguished from those from wastewater treatment works receiving a high industrial influent load, and there was considerable uniformity across sites in terms of toxicity. Industrial effluents were less toxic than municipal ones. The sampling schedule recommended by the toolkit was reasonable given the results obtained, and test response to industrial effluent was more even than in other sectors. Given the potential variability in industrial effluent chemistry, a wide range of test taxa is recommended for ongoing use.

Mining effluent was less toxic than the above effluents. A problem associated with some mines with major implications for water quality in South Africa is acid mine drainage (AMD) (CSIR 2010; DWA 2011; Expert Team of the Inter-Ministerial Committee under the Coordination of the Council for Geoscience 2010; McCarthy, 2011). Consequences for water receiving acid mine drainage, even if treated, often include high salinity and potentially toxic metal loads. The mining effluent assessed during this project did not show any indications, such as low pH or high salinity, that might indicate acid mine drainage. As a result, the mining results reported here cannot be said to represent the impact of treated or untreated acid mine drainage. The relatively low toxicity of mining effluent was something of a surprise, and at some (though not all) of the sampled points the toxicity of the effluent was comparable to, and sometimes better than, the resource. Given the potential for mining impacts on water it is recommended that the sampling frequency be maintained at the rate recommended by the IWUAB toolkit. Test response to effluent from this sector was comparable except for bacteria, which were stimulated by the effluent and water from the resource.

There was no effluent associated with agricultural sites surveyed for this study. All assessments were made in terms of upstream and downstream samples of resource water. No increase in resource toxicity as a result of agricultural activity was found. Given that the sites were drawn from sites with irrigated agriculture and one feedlot, these can be taken as potentially indicative of a broad range of agriculture in the country. However, no known spraying or biocide use was reported. As biocides can be detected even when no spraying occurs (du Preez et al., 2005), and samples were collected from areas that experience biocide use (Dabrowski, 2015), exposure to biocides cannot be ruled out. Biocides require a great deal of dilution in the resource to reach safer levels (Dabrowski et al., 2009). As the major impacts of farming on water quality are eutrophication and biocides (Dabrowski et al., 2009), and eutrophication at lower levels should not lead to a toxic response, it is likely that at the scale of a single farm that the major concern would be biocide use. Biocide use may explain the upstream resource toxicities found in rural areas (some rivers that were sampled drain from urban or mining regions where other impacts are anticipated).For this reason, it is important that when biocide application occurs, sampling should be scheduled around it. Recommendations for timing and frequency of sampling are also likely to vary in a location-specific way. It may be of value for the IWUAB toolkit to provide a list or indication of proscribed activities that would require sampling.

In many cases, it may be possible to reduce the number of tests used provided that enough sensitive tests are retained and a range of trophic levels are considered. It may also be possible to change the sampling frequency from those produced by the IWUAB toolkit. The data presented here may give some guidelines in this regard, but site-specific recommendations would be best made once at least two years of data from a particular site have been collected and assessed.

4.1.2 Effluent toxicity and the resource

It is important to note that while application of toxicity testing in managing the quality of the resource is to be welcomed, the application of toxicity testing alone is insufficient to manage resource quality. Application of toxicity testing of effluent should be accompanied by appropriate chemical monitoring, and toxicity testing of the resource should be accompanied by a chemical testing programme together with an appropriate biomonitoring programme. The three approaches used together provide a sufficient and appropriate toolkit for resource quality monitoring and management.

Results from the project demonstrated that very toxic effluent could be found to have no detectable impact on the resource, and that the times that a clear effluent effect could be demonstrated were relatively rare. In addition, variations in effluent and resource toxicity were found that could not be linked to any clear cause. As a result, attempts to statistically establish an effluent impact on the resource were not successful. The lack of statistical support is also linked to the relatively small number of sites sampled. However, a large sample that may have established statistical trends would have had prohibitive costs attached and was beyond the scope of the current study.

Despite difficulty in establishing clear effluent-driven changes in resource toxicity, some extremely toxic effluents were encountered. For the worst example, municipal effluent, which comprises a varying mix of treated domestic sewage and industrial waste, was capable of killing all of at least one test taxon was found 57% of the time, and, of these, 53% killed all test taxa. Other sectors were better, although all sectors occasionally produced effluent that was capable of killing all of all least one of the test taxa. This does not include agriculture, as none of the sites surveyed had any effluent release. In the light of these highly toxic effluents, not finding a clear signature of a change in effluent toxicity was a surprise.

Simultaneously, although statistically significant effluent impact in any sector could not be demonstrated, resource toxicity precluded the application of one of the compliance guidelines proposed for the IWUAB toolkit, namely that downstream samples at all times should be at a level such that an effect level (here mortality) of less than 50% in any test taxon was found. The reason for this was that upstream samples were frequently more toxic than this level already, and without any demonstrated effluent impact, the compliance guidelines were exceeded. Relatively few upstream samples (6%) were found to have no detectable toxic effects whatsoever. This renders the compliance criteria potentially legally indefensible, but also demonstrates a high upstream toxicity before any additional impact. In the absence of further analysis, the cause of the upstream toxicity is not known. However, this paints a disturbing picture of resource toxicity in the area sampled by this project. It is not clear whether the frequently high effluent toxicities encountered during this project may have had a cumulative effect on resource toxicity, even when individual effluents assessed alone often had no demonstrated impact.

4.1.3 Seasonality of responses

Despite seasonal flow pattern changes experienced in the area of study, no clear seasonal change in resource response to point or diffuse effluent could be found. Although lower flow rates in the dry season may be expected to lower the dilution capacity of the resource and thereby increase toxicity of the resource in response to impacts, no such change was found. Effluent toxicities varied throughout the year with no clear pattern in any sector, and this may have reduced overt trends in response to resource dilution capacity.

4.1.4 Location of sampling sites

Details on sampling recommendations regards timing and appropriate analyses are discussed above. Here specifics regarding the location of sampling points are considered. The IWUAB guideline has some information on sampling point selection, and considers, without detailed recommendations, how these should be placed so as to be close enough to represent an impact adequately, but should be placed in such a way that a single impact only is considered. For the most part, this should be relatively straightforward to achieve. Two cases arise where this will be less easily achieved.

Sampling needs to be placed such that other potential conflicting impacts are avoided. This is generally straightforward where point source release is considered. However, this is much less likely where diffuse release of effluents is considered, as is common in the agricultural sector. Where effluent release is diffuse, the distance between clear upstream and downstream sites is larger and it is more likely that another potential impact might lie between the upstream and downstream points. Again, using the agricultural sector, this could be another farm, with a separate water use authorisation, on the opposite side of a receiving water body. The IWUAB toolkit should make further recommendations as to how sampling should be undertaken in these circumstances. This could be in a guideline document, or on output from the IWUAB toolkit itself. If the former, the IWUAB toolkit output should refer to the guideline document for sampling recommendations.

Another case where upstream and downstream sampling cannot simply be undertaken would occur where there is no water at the upstream site. This was indicated as likely in workshops on the toolkit and was encountered during the course of this project. This has several consequences. The first is given the lack of upstream water to dilute the effluent stream, the flow in the resource is effectively all effluent and the results of tests from effluent and downstream samples should be comparable. It also has the result that compliance criteria cannot be phrased in terms of comparison of upstream and downstream as is currently the case in IWUAB output. Despite the problems identified with use of blanket downstream compliance condition that are documented above, in this case compliance criteria recommendations based on effluent or downstream samples alone may be required, or recommended river state phrased in RQOs or possibly PES/REC.

4.1.5 DWS and CMA input and buy-in

There is on the face of it little refinement that the IWUAB toolkit requires. The tests that are recommended build on a long period of research on appropriate methodologies and on international experience in management of discharged effluent. The means of combining test results and classifying the output is reasonable and liable to be acceptable to managers. The main stumbling block is linked to compliance recommendations generated by the kit.

In contacts with DWS staff at workshops where the IWUAB toolkit was presented or discussed, several general responses to the toolkit were received:

- The potential value of the toolkit was recognised in overcoming lack of toxicological capacity at the regulator potentially leading to its appropriate application in water use authorisation.
- The question of using the toolkit to suggest or define compliance criteria for monitoring was questioned. This was stressed in the light of the current suggested criterion that downstream toxicity be at all times maintained at a hazard class of below class three at all times, regardless of the upstream hazard classification. The legal indefensibility of the criterion was noted. Several people proposed that in the light of this, the toolkit would best be deployed in a screening role and not used to recommend compliance terms.
- Despite the input of DWS staff at several points during the work leading to the production of the IWUAB toolkit, several staff expressed surprise at the existence of the toolkit and questioned how it had been designed without known, planned DWS input.

In short, the value of the toolkit was recognised as a tool to aid in the effective implementation of toxicological testing in effluent management and as an aid to SDC implementation. However, there was some scepticism as to its anticipated role and particular to its use in defining compliance criteria. The classification system that the toolkit uses to combine toxicological test results to give an ecologically relevant output classification (Persoone et al., 2003) is already used in the NTMP (D Odusanya pers. comm.). Its value to managers is apparent in its generation of ranked classes, which have an easily interpreted and ecologically meaningful output. The toxicological classification used in IWUAB recommendations is an therefore an appropriate and comprehensible tool use in summarizing test results, and one that already sees some use in NTMP.

The above points indicate a wariness about adopting a toolkit that may improve the water use authorisation process but is perceived not to be produced in collaboration with DWS, and a greater wariness about accepting unsolicited external recommendations in the water use authorisation process. Both of these broad points will hinder routine application of the toolkit at DWS. The use of the toolkit as a screening tool was seen as more palatable by some DWS staff.

In order that the toolkit be more widely acceptable and to be used to facilitate the adoption of toxicological testing in the water use authorisation process including its contributed proposed compliance criteria, it is recommended the issues related to finalization of the IWUAB toolkit to DWS (and CMA) requirements be resolved in a formal engagement process with the regulators. Such a process will improve regulatory familiarity with the toolkit, will ensure that finalizations meet the regulator's requirements, and will engender a greater sense of ownership and buy-in into the project.

As demonstrated above, one of the suggested criteria, which applies a blanket rule to permissible downstream toxicity without relating this to upstream toxicity, is legally indefensible. The other criterion, that the hazard class should not increase by more than one unit from upstream to downstream, seems reasonable. However, in order that suggested criteria are acceptable to the regulator, it is important that the reformulation of suggested compliance criteria be undertaken in collaboration with the regulator. Criteria may also include references to effluent toxicity, which is currently not used by the IWUAB toolkit. The engagement process may also involve identifying whether application of the IWUAB toolkit and toxicological sampling should be involved in all water use authorisations, and if not, then appropriate cases or conditions need to be identified.

Engagement with the regulator will need to involve decision-makers of sufficient seniority or authority. These should primarily be drawn from staff engaged with the licensing process, from the NTMP, and other water quality managers. Engagement will ideally include appropriate staff from the operational CMAs. In addition, legal input to determine the defensibility of any suggested compliance criteria would be required. Consideration of use of class weight scores in conjunction with hazard classes, as proposed in Persoone et al. (2003) might also be considered.

4.2 RECOMMENDATIONS

The IWUAB toolkit is a valuable tool to aid in implementing valid use of toxicological in effluent and water quality management. It provides suggestions of appropriate tests per sector depending on user input, and follows international best practice in recommending a test battery for whole effluent testing. It supports a method that combines the output from multiple tests to provide an ecologically meaningful ranked classification of toxic hazard, and which is capable of utilizing the output from screening as well as definitive tests. It generates a sampling scheme for sample collection.

However, there are some identified issues that need to be addressed prior to finalization. All are addressed in greater detail above:

- Package finalization should be undertaken in a process that engages DWS staff. The recommended compliance criteria were highlighted as an area that needs rethought. This process should engage legal input in order to produce legally defensible compliance criteria. Engaging DWS staff with appropriate seniority will aid in adoption and utilization of the IWUAB toolkit.
- Sampling recommendations need to be assessed to more completely consider how to address conflicting impacts, in particular where diffuse release of effluent is present. This process should be undertaken with DWS input.

- Sampling frequency recommendations need to be clarified (in particular for agriculture). Stipulations on modifying sampling regimes based on assessment of collected site-specific data need to be specifically addressed.
- Recommendations regarding selection of bioassays should be reassessed in light of data presented here.
- The potential of setting criteria for effluent (rather than the resource) needs reconsideration.
- DWS need to be engaged regards storage of data generated by the IWUAB toolkit and its application in Water Use Licensing.
- Other issues identified in the text above should be addressed.
- Once the IWUAB toolkit has been finalized to a level required by DWS, it should be adopted to provide support for the use of toxicological testing in Water Use Licensing in order that all tools to appropriately manage resource quality are in use.

REFERENCES

AGUNBIADE, F.O. and MOODLEY, B. (2015) Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, Kwazulu-Natal, South Africa. *Environmental Chemistry* 35(1): 36-46.

ASHTON, P.J. and DABROWSKI, J.M. (2011) *An overview of surface water quality in the Olifants River catchment*. WRC Report No. KV 293/11. Water Research Commission, Gezina.

BATES, D., MAECHLER, M., BOLKER, B. and WALKER, S. (2013) *lme4: Linear mixed-effects models using Eigen and S4*. R package version 1.0-5. http://CRAN.R-project.org/package=lme4.

BAUDOIN, M-A., VOGEL, C., NORTJE, K. and NAIK, M. (2017) Living with drought in South Africa: lessons learnt from the recent El Niño drought period. *International Journal of Disaster Risk Reduction* 23: 128-137.

BOX, G.E.P. and COX, D.R. (1964) An analysis of transformations. *Journal of the Royal Statistical Society, Series B* 26: 211-252.

CALOW, P. (1998) Ecological Risk Assessment: Risk for What? How Do We Decide? *Ecotoxicology and Environmental Safety* 40: 15-18.

CHAPMAN, A.A., VENTER, E.A. and PEARSON, H. (2011a) *Aquatic toxicity testing in South Africa: Status of aquatic toxicity testing in South Africa.* WRC Report No. 1853/1/11. Water Research Commission, Gezina.

CHAPMAN, A.A., VENTER, E.A. and PEARSON, H. (2011b) *Aquatic toxicity testing in South Africa: guideline for the accreditation of routine aquatic testing laboratories.* WRC report No. TT504/11. Water Research Commission, Gezina.

CHAPMAN, P.M. (2000) Whole effluent toxicity testing – usefulness, level of protection, and risk assessment. *Environmental Toxicology and Chemistry* 19(1): 3-13.

CHAPMAN, P.M. (2007) Determining when contamination is pollution – Weight of evidence determinations for sediments and effluents. *Environment International* 33: 492-501.

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH (CSIR) (2010) *A CSIR perspective on water in South Africa – 2010*. CSIR Report No. CSIR/NRE/PW/IR/2011/0012/A. Council for Scientific and Industrial Research, Pretoria.

DABROWSKI, J.M., MURRAY, K., ASHTON, P.J. and LEANER, J.J. (2009) Agricultural impacts on water quality and implications for virtual water trading decisions. *Ecological Economics* 68: 1074-1082.

DABROWSKI, J.M. (2015) Development of pesticide use maps for South Africa. *South African Journal of Science*:111(1/2), Art. #2014-0091.

DAVIS-REDDY, C.L. and VINCENT, K. (2017) *Climate risk and vulnerability: A handbook for Southern Africa (2nd ed.).* Council for Scientific and Industrial Research, Pretoria.

DE MENDIBURU, F. (2017). *agricolae: Statistical Procedures for Agricultural Research.* R package version 1.2-8. https://CRAN.R-project.org/package=agricolae.

DEPARTMENT OF WATER AFFAIRS (DWA) (2011) *Planning level review of water quality in South Africa.* Sub-series No. WQP 2.0. Department of Water Affairs, Pretoria.

DEPARTMENT OF WATER AFFAIRS (DWA) (2013) *National Water Resource Strategy 2nd edition.* Department of Water Affairs, Pretoria.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (1996) *South African water quality guidelines. Volumes 1-8*. Department of Water Affairs and Forestry, Pretoria.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (2003) *The management of complex industrial wastewater discharges. Introducing the Direct Estimation of Ecological Effect Potential (DEEEP) approach. A discussion document*. Institute for Water Quality Studies, Department of Water Affairs and Forestry, Pretoria.

DEPARTMENT OF WATER AFFAIRS AND FORESTRY (DWAF) (2005) *National Toxicity Monitoring Programme for Surface Waters: Draft Conceptual Design Framework and Record of Decision Report*. Department of Water Affairs and Forestry, Pretoria.

DIAMOND, J. and DALEY, C. (2000) What is the relationship between Whole Effluent Toxicity and instream biological condition? *Environmental Toxicology and Chemistry* 19(1): 158-168.

DU PREEZ, L.H., JANSEN VAN RENSBURG, P.J., JOOSTE, A.M., CARR, J.A., GIESY, J.P., GROSS, T.S., KENDALL, R.J., SMITH, E.E., VAN DER KRAAK, G. and SOLOMON, K.R. (2005) Seasonal exposures to triazine and other pesticides in surface waters in the western Highveld corn-production region in South Africa *Environmental Pollution* 135: 131-141.

ECONOMIC DEVELOPMENT DEPARTMENT (EDD) (2011) *The New Growth Path: Framework.* Economic Development Department, Pretoria.

EXPERT TEAM OF THE INTER-MINISTERIAL COMMITTEE UNDER THE COORDINATION OF THE COUNCIL FOR GEOSCIENCE (2010) *Mine water management in the Witwatersrand gold fields with special emphasis on acid mine drainage*. Report to the Inter-Ministerial Committee on Acid Mine Drainage.

FOX, J. and WEISBERG, S. (2011) *An R companion to applied regression, second edition*. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.

GRIFFIN, N.J., MULLER, W.J. and GORDON, A.K. (2011) *Implementation of ecological hazard assessment of industrial waste discharge: a comparison of toxicity test methods*. WRC Report No. KV 276/11. Water Research Commission, Gezina.

GRIFFIN, N.J., PALMER, C.G. and SCHERMAN, P-A. (2014) *Critical analysis of environmental water quality in South Africa: historic and current trends*. WRC Report No. 2184/1/14. Water Research Commission, Gezina.

HAIGH, E.H. and DAVIES-COLEMAN, H.D. (1999) *The development of a production facility for standard laboratory test organisms for ecotoxicological research*. WRC Report No. 755/1/99. Water Research Commission, Gezina.

HARDING, W.R. (2015) Living with eutrophication in South Africa: a review of realities and challenges. *Transactions of the Royal Society of South Africa* 70(2): 155—171.

HE, L-Y., YING, G-G., LIU, Y-S., SU, H-C., CHEN, J., LIU, S-S. and ZHAO, J-L. (2016) Discharge of swine wastes risks water quality and food safety: Antibiotics and antibiotic resistance genes from swine sources to the receiving environments. *Environment International* 92-93: 210-219.

HUIZENGA, J.M. (2011) Characterisation of the inorganic chemistry of surface waters in South Africa. *Water SA* 37(3): 401-410.

HUNTER, C.H., SENIOR, E., HOWARD, J.R. and BAILEY, I.W. (1997) *Development of a multi-stage laboratory model to determine the ecotoxicological impacts of selected xenobiotic compounds*. WRC Report No. 583/1/97. Water Research Commission, Gezina.

JONKER, M-N., VAN VUREN, J.H.J. and WEPENER, V. (2009) The impact of feedlot effluent on water quality and aquatic macroinvertebrate community structure in streams of the upper Vaal River catchment, South Africa. *African Journal of Aquatic Science* 34(3): 219-230.

JOOSTE, S. and SLABBERT, L. (2006) Experience from the testing of the DEEEP methodology: Preliminary results. *Proceeding of the WISA conference held in Durban 21-24 May 2006*. Water Institute of Southern Africa, Midrand.

KLEYNHANS, C.J. and LOUW, M.D. (2008) *Module A: EcoClassification and EcoStatus determination in River EcoClassification: Manual for EcoStatus Determination (version 2)*. Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC Report No. TT 329/08. Water Research Commission, Gezina.

LEET, J.K., LEE, L.S., GALL, H.E., GOFORTH, R.R., SASSMAN, S., GORDON, D.A., LAZORCHAK, J.M., SMITH, M.E., JAFVERT, C.T. and SEPÚLVEDA, M.S. (2012) Assessing impacts of land-applied manure from concentrated animal feeding operations on fish populations and communities. *Environmental Science and Technology* 46(24): 13440-13447.

MANICKUM, T. and JOHN, W. (2014) Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa). *Science of The Total Environment* 468-469: 584-597.

MCCARTHY, T.S. (2011) The impact of acid mine drainage in South Africa. *South African Journal of Science* 107(5/6): Art. #712.

MERCHÁN, D., CASALÍ, J., DEL VALLE DE LERSUNDI, J., CAMPO-BESCÓS, M.A., GIMÉNEZ, R., PRECIADO, B. and LAFARGA, A. (2018) Runoff, nutrients, sediment and salt yields in an irrigated watershed in southern Navarre (Spain). *Agricultural Water Management* 195: 120-132.

MIYASHIRO, T. and RUBY, E.G. (2012) Shedding light on bioluminescence regulation in *Vibrio fischeri*. *Molecular Microbiology* 84(5): 795-806.

MOLOKWANE, P.E., MELI, K.C. and NKHALAMBAYAUSI-CHIRWA, E.M. (2008) Chromium (VI) reduction in activated sludge bacteria exposed to high chromium loading: Brits culture (South Africa). *Water Research* 42(17): 4538-4548.

MORTENSEN, J.G., GONZÁLEZ-PINZÓN, R., DAHM, C.N., WANG, J., ZEGLIN, L.H. and VAN HORN, D.J. (2016) Advancing the Food-Energy-Water Nexus: Closing Nutrient Loops in Arid River Corridors. *Environmental Science and Technology* 50(16): 8485-8496.

NEL, J.L., DRIVER, A., STRYDOM, W.F., MAHERRY, A., PETERSEN, C., HILL, L., ROUX, D.J., NIENABER, S., VAN DEVENTER, H., SWARTZ, E. and SMITH-ADAO, L.B. (2011a) *Atlas of freshwater ecosystem priority areas in South Africa: Maps to support sustainable development of water resources*. WRC Report No. TT 500/11. Water Research Commission, Gezina.

NEL, J.L., MURRAY, K.M., MAHERRY, A.M., PETERSEN, C.P., ROUX, D.J., DRIVER, A., HILL, L., VAN DEVENTER, H., FUNKE, N., SWARTZ, E.R., SMITH-ADAO, L.B., MBONA, N., DOWNSBOROUGH, L. and NIENABER, S. (2011b) *Technical Report for the National Freshwater Ecosystem Priority Areas project*. WRC Report No. 1801/2/11. Water Research Commission, Gezina.

NEWMAN, M.C., OWNBY, D.R., MEZIN, L.C.A., POWELL, D.C., CHRISTENSEN, T.R.L., LERBERG, S.B. and ANDERSON, B-A. (2000) Applying species-sensitivity distribution in ecological risk assessment: assumptions of distribution type and sufficient numbers of species. *Environmental Toxicology and Chemistry* 19(2): 508-515.

PALMER, C.G., ROSSOUW, N., MULLER, W.J. and SCHERMAN, P-A. (2005) The development of water quality methods within ecological Reserve assessments, and links to environmental flows. *Water SA* 31(2): 161-170.

PEARSON, H., SHADDOCK, B.F., MULDER, P.F.S. and CLOETE, Y.C. (2015) *Development of research support to enable the issuing of aquatic toxicity based water use licenses: Guideline document.* WRC Report No. KV 347/15. Water Research Commission, Gezina.

PERSOONE, G., MARSALEK, B., BLINOVA, I., TÖRÖKNE, A., ZARINA, D., MANUSADZIANAS, L., NALECZ-JAWECKI, G., TOFAN, L., STEPANOVA, N., TOTHOVA, L. and KOLAR, B. (2003) A practical and user-friendly toxicity classification system with Microbiotests for natural waters and wastewaters. *Environmental Toxicology* 18: 395-402.

POWER, E.A. and BOUMPHREY, R.S. (2004) International trends in bioassay use for effluent management. *Ecotoxicology* 13: 377-398.

R CORE TEAM (2017) *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RIPLEY, B.D. and LAPSLEY, M. (2017) *RODBC: ODBC Database Access*. R package version 1.3-15. https://CRAN.R-project.org/package=RODBC

SCHERMAN, P-A., PALMER, C.G. and MULLER, W.J. (2003) *Use of indigenous riverine invertebrates in applied toxicology and water resource quality management*. WRC Report No. 955/1/03. Water Research Commission, Gezina.

SCHERMAN, P-A., MULLER, W.J. and JOOSTE, S. (2004) *A further investigation into using a risk-based approach for setting integrated environmental objectives for the protection of water resources*. WRC Report KV 154/04. Water Research Commission, Gezina.

SLABBERT, J.L. (1998) *Guidelines for toxicity bioassaying of waters and effluents in South Africa*. WRC Report No. 358/1/98. Water Research Commission, Gezina.

SLABBERT, J.L. and MURRAY, K. (2011) *Guidance for the selection of toxicity tests in support of the information requirements of the National Water Act*. WRC Report No 1211/1/10. Water Research Commission, Gezina.

SLABBERT, L. (2004) *Methods for direct estimation of ecological effect potential (DEEEP).* WRC Report No. 1313/1/04. Water Research Commission, Gezina.

THAMAGA-CHITJA, J.M. and MOROJELE, P. (2014) The context of smallholder farming in South Africa: towards a livelihood asset building framework. *Journal of Human Ecology* 45(2): 147-155.

TONKES, M. and BALTUS, C.A.M. (1997) *Praktijk onderzoek aan complexe effluenten met de Totaal Effluent Milieubezwaarlijkheid (TEM) methodiek. Resultaten van 10 complexe effluenten*. RIZA-rapportnummer 97.033. Institute of Inland Water Management and Waste Water, Treatment, Lelystad.

TONKES, M., DE GRAAF, P.J.F. and GRAANSMA, J. (1999) Assessment of complex industrial effluents in the Netherlands using a Whole Effluent Toxicity (or WET) approach. *Water Science and Technology* 39(10-11): 55-61.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) (2004) *National Whole Effluent Toxicity (WET) implementation guidance under the NPDES Program (draft)*. Office of Wastewater Management, United States Environmental Protection Agency, Washington.

VENABLES, W.N. and RIPLEY, B.D. (2002) *Modern applied statistics with S. Fourth edition*. Springer, New York. ISBN 0-387-95457-0.

WICKHAM, H. (2007) Reshaping data with the reshape package. *Journal of Statistical Software* 21(12): 1-20. URL http://www.jstatsoft.org/v21/i12/.

WICKHAM, H. (2009) *ggplot2: elegant graphics for data analysis*. Springer, New York.

WICKHAM, H. (2011) The Split-Apply-Combine strategy for data analysis. *Journal of Statistical Software* 40(1): 1-29.

ZUUR, A.F., IENO, E.N., WALKER, N.J., SAVELIEV, A.A., and SMITH, G.M. (2009) *Mixed effects models and extensions in ecology with R.* Springer, New York.

APPENDIX A: DETAILED HAZARD CLASSES AND CLASS WEIGHT SCORES

All hazard classes and class weight scores collected are presented in Table 3. Actionable results recommended by the IWUAB are indicated in the table.

Table 3: Hazard classes, class weight scores, and IWUAB actionable results for upstream, downstream, and effluent samples from all sample visits at all sites in each sector.

			Hazard class			Weight score			
Sector	**Site**	**Sampling date**	**Upstream**	**Effluent**	**Downstream**	**Upstream**	**Effluent**	**Downstream**	**IWUAB action**
Agriculture	37218	December 2016	3		2	25		25	
		April 2017	2		2	75		75	
		August 2017	3		2	50		75	
		October 2017	3		3	38		38	DHC>2
	48921	December 2016	3		3	25		25	DHC>2
		April 2017	2		2	25		50	
		August 2017	2		2	25		50	
		October 2017	3		3	63		50	DHC>2
	71057	December 2016							
		April 2017	2		2	75		50	
		August 2017	2		2	75		50	
		October 2017	3		3	38		38	DHC>2
	72621	December 2016	3		2	25		25	
		April 2017	2		2	25		50	
		August 2017	2		2	25		50	
		October 2017	3		3	25		38	DHC>2
	74397	December 2016	2		2	25		50	
		April 2017	2		2	25		50	
		August 2017	3		2	63		50	
		October 2017	3		3	38		63	DHC>2
Industry	12315	April 2017	2	4	2	50	67	50	
		July 2017	3	2	2	50	25	75	
		October 2017	2	2	2	50	25	75	
		February 2018	2	2	2	25	50	25	

Sector	Site	Sampling date	Hazard class: Upstream	Hazard class: Effluent	Hazard class: Downstream	Weight score: Upstream	Weight score: Effluent	Weight score: Downstream	IWUAB action
Industry	23713	April 2017	2	2	2	50	75	75	
		July 2017	3	3	2	50	38	50	
		October 2017							
		February 2018	2	4	2	50	75	50	
	35546	April 2017	3	4	2	63	83	75	
		July 2017	2	3	3	50	50	50	DHC>2
		October 2017							
		February 2018	2	2	3	25	50	38	DHC>2
	47335	April 2017	2	3	2	50	63	75	
		July 2017	2	3	2	25	50	50	
		October 2017	2	3	3	50	63	63	DHC>2
		February 2018		2	2		50	50	
	84187	April 2017	3	2	2	50	25	75	
		July 2017	4	3	3	58	50	50	DHC>2
		October 2017							
		February 2018	2	4	2	50	42	50	
Mining	7779	October 2016	2	3	2	25	50	25	
		January 2017	3	2	2	50	50	75	
		April 2017	2	2	2	50	75	50	
		July 2017		3	3		38	38	DHC>2
	14555	October 2016	2	3	2	25	38	75	
		January 2017	4	2	2	50	75	75	
		April 2017	2	3	2	75	75	25	
		July 2017	2	2	2	75	50	50	
	16962	October 2016	3	3	3	50	50	50	DHC>2
		January 2017	3	3	3	63	63	50	DHC>2
		April 2017	2	3	2	50	38	25	
		July 2017	3	3	2	38	63	50	
	27186	October 2016	2	3	2	50	38	50	
		January 2017	3	2	2	63	50	75	
		April 2017	2	3	2	50	50	50	
		July 2017	2	4	3	50	42	38	DHC>2
	91444	October 2016	3	2	3	38	25	25	DHC>2
		January 2017	3	2	2	38	75	100	
		April 2017	2	2	3	75	75	38	DHC>2
		July 2017	2	2	2	25	50	50	
Municipal	43114	September 2016	1	5	1	0	100	0	
		November 2016	2	5	2	33	100	100	

			Hazard class			Weight score			
Sector	**Site**	**Sampling date**	**Upstream**	**Effluent**	**Downstream**	**Upstream**	**Effluent**	**Downstream**	**IWUAB action**
Municipal	43114	January 2017	2	2	3	75	50	62	DHC>2
		March 2017	2	3	2	33	67	33	
		May 2017	2	2	2	33	33	33	
		July 2017	3	5	3	50	100	67	DHC>2
	49909	September 2016	1	5	2	0	100	33	
		November 2016	2	4	3	100	33	50	DHC>2
		January 2017	2	2	2	50	25	75	
		March 2017	2	2	2	33	67	33	
		May 2017	3	4	2	33	67	33	
		July 2017	2	4	4	33	33	44	HCD>1, DHC>2
	64033	September 2016	1	2	1	0	67	0	
		November 2016	2	4	2	67	56	67	
		January 2017	3	3	2	38	75	25	
		March 2017	2	5	2	33	100	67	
		May 2017	2	5	4	67	100	78	HCD>1, DHC>2
		July 2017	2	5	4	33	100	89	HCD>1, DHC>2
	71056	September 2016	2	4	2	33	67	33	
		November 2016	2	2	2	67	33	67	
		January 2017	3	2	2	50	50	50	
		March 2017	1	2	2	0	33	33	
		May 2017	2	4	2	67	89	67	
		July 2017	2	1	2	67	0	33	
	75130	September 2016	1	5	2	0	100	33	
		November 2016	2	2	2	67	33	67	
		January 2017	2	4	3	25	92	50	DHC>2
		March 2017	2	4	2	33	78	33	
		May 2017	2	3	3	33	33	50	DHC>2
		July 2017	2	5	2	33	100	33	

DHC>2: Downstream hazard class is three or greater.

HCD>1: Hazard class increased by more than one unit from upstream to downstream.

APPENDIX B: INHIBITION AND STIMULATION IN RESPONSE TO EFFLUENT EXPOSURE

The percentage inhibition/mortality or stimulation experience by test taxa in each of the tests of each sample collected during the project is presented below in Table 4 below. Data are derived from stimulation and inhibition data from each screening test combined with inhibition and stimulation rates from definitive tests in 100% effluent. Note that the tests undertaken varied occasionally depending on IWUAB recommendations.

Table 4: Inhibition/mortality (-ve) or stimulation (+ve) at end of test in full strength effluent. Data from screening tests and definitive tests are combined. All data expressed as a percentage.

				Inhibition (-)/stimulation (+)		
Sector	**Site**	**Date**	**Test**	**Upstream**	**Effluent**	**Downstream**
Agriculture	37218	December 2016	Algae	-72		15
			Bacteria	0		-27
			Crustacea	7		0
			Fish	0		0
		April 2017	Algae	10		25
			Bacteria	-25		-29
			Crustacea	-30		-25
			Fish	-10		-10
		August 2017	Algae	18		-26
			Bacteria	-30		-25
			Crustacea	-10		0
			Fish	-63		-13
		October 2017	Algae	-8		3
			Bacteria	-52		-56
			Crustacea	-10		0
			Fish	0		-20
	48921	December 2016	Algae	-65		-73
			Bacteria	-2		-12
			Crustacea	0		0
			Fish	0		0
		April 2017	Algae	25		-1
			Bacteria	-15		24
			Crustacea	-20		-10
			Fish	0		0
		August 2017	Algae	60		62
			Bacteria	-28		-29

Sector	Site	Date	Test	Upstream	Effluent	Downstream
				Inhibition (-)/stimulation (+)		
Agriculture	48921	August 2017	Crustacea	-5		0
			Fish	0		-13
		October 2017	Algae	0		-50
			Bacteria	0		-50
			Crustacea	0		0
			Fish	0		0
	71057	December 2016	Algae	0		-10
			Bacteria	0		-1
			Crustacea	0		0
			Fish	0		0
		April 2017	Algae	21		-18
			Bacteria	-20		-15
			Crustacea	-25		-10
			Fish	-20		-10
		August 2017	Algae	12		8
			Bacteria	-25		-20
			Crustacea	-20		0
			Fish	-38		-10
		October 2017	Algae	-72		-52
			Bacteria	-1		-49
			Crustacea	0		0
			Fish	-10		0
	72621	December 2016	Algae	-64		-44
			Bacteria	-5		0
			Crustacea	0		-2
			Fish	0		0
		April 2017	Algae	0		-17
			Bacteria	-14		-19
			Crustacea	-15		-20
			Fish	0		-10
		August 2017	Algae	43		60
			Bacteria	-22		-28
			Crustacea	0		-5
			Fish	0		0
		October 2017	Algae	0		-4
			Bacteria	0		-55
			Crustacea	0		-5
			Fish	0		-10
	74397	December 2016	Algae	63		-33
			Bacteria	-23		-24
			Crustacea	0		0
			Fish	0		0
		April 2017	Algae	28		40

				Inhibition (-)/stimulation (+)		
Sector	**Site**	**Date**	**Test**	**Upstream**	**Effluent**	**Downstream**
Agriculture	74397	April 2017	Bacteria	-6		-15
			Crustacea	-20		-40
			Fish	0		-10
		August 2017	Algae	-28		7
			Bacteria	-25		-19
			Crustacea	-10		-10
			Fish	-50		-13
		October 2017	Algae	-4		-65
			Bacteria	-51		-53
			Crustacea	-20		-10
			Fish	0		0
Industry	12315	April 2017	Algae	11	-66	2
			Bacteria	5	-53	-1
			Crustacea	-25	-20	-25
			Fish	-30	-100	-10
		July 2017	Algae	0	-3	-29
			Bacteria	0	-26	-26
			Crustacea	0	0	-5
			Fish	0	0	-25
		October 2017	Algae	-44	-15	-35
			Bacteria	-24	-47	-27
			Crustacea	-5	-5	0
			Fish	0	0	-10
		February 2018	Algae	13	-37	4
			Bacteria	-46	-30	-47
			Crustacea	-5	0	0
			Fish	0	0	0
	23713	April 2017	Algae	-8	0	44
			Bacteria	-25	-23	-30
			Crustacea	0	-10	-15
			Fish	-10	-30	0
		July 2017	Algae	-26	-27	-9
			Bacteria	33	35	34
			Crustacea	-70	-90	-15
			Fish	-40	0	-20
		February 2018	Algae	-5	51	36
			Bacteria	-46	-68	-48
			Crustacea	-10	-100	-25
			Fish	0	-60	0
	35546	April 2017	Algae	-93	-99	-6
			Bacteria	-39	-88	-27
			Crustacea	-10	-100	-30
			Fish	-20	-100	-10

Sector	Site	Date	Test	Inhibition (-)/stimulation (+) Upstream	Effluent	Downstream
Industry	35546	July 2017	Algae	68	-99	-95
			Bacteria	38	34	36
			Crustacea	-30	-25	-30
			Fish	-20	-13	-20
		February 2018	Algae	37	30	40
			Bacteria	-49	-47	-51
			Crustacea	0	-30	-10
			Fish	0	0	0
	47335	April 2017	Algae	5	-22	-35
			Bacteria	-42	-46	-39
			Crustacea	-25	-45	-35
			Fish	0	-50	0
		July 2017	Algae	19	6	-6
			Bacteria	-23	-20	-24
			Crustacea	0	-90	0
			Fish	0	-10	-13
		October 2017	Algae	-32	-60	-92
			Bacteria	-17	-49	-40
			Crustacea	0	-10	-10
			Fish	-10	-30	-10
		February 2018	Algae	0	6	28
			Bacteria	0	-22	-47
			Crustacea	0	-10	-10
			Fish	0	0	0
	84187	April 2017	Algae	6	26	2
			Bacteria	-46	7	-40
			Crustacea	-45	-15	-30
			Fish	-50	0	-30
		July 2017	Algae	-45	-63	10
			Bacteria	23	-38	43
			Crustacea	-100	0	-80
			Fish	-100	-13	-80
		February 2018	Algae	6	25	48
			Bacteria	-45	-53	-49
			Crustacea	-15	-100	-20
			Fish	0	0	0
Mining	7779	October 2016	Algae	11	-90	-13
			Bacteria	25	26	31
			Crustacea	-35	-45	-35
			Fish	0	-10	0
		January 2017	Algae	-13	-11	-6
			Bacteria	-27	-8	-26
			Crustacea	-55	-30	-40

Sector	Site	Date	Test	Inhibition (-)/stimulation (+) Upstream	Effluent	Downstream
Mining	7779	January 2017	Fish	-30	-10	-20
		April 2017	Algae	1	-41	18
			Bacteria	-5	3	-4
			Crustacea	-20	-10	-25
			Fish	-20	-30	-40
		July 2017	Algae	0	25	-63
			Bacteria	0	40	42
			Crustacea	0	-15	0
			Fish	0	-50	-20
	14555	October 2016	Algae	-10	-82	-10
			Bacteria	33	16	27
			Crustacea	-30	-10	-40
			Fish	0	0	-10
		January 2017	Algae	-38	-31	-37
			Bacteria	-38	-9	-20
			Crustacea	-100	-45	-40
			Fish	-30	-20	0
		April 2017	Algae	-33	-83	1
			Bacteria	-1	1	-2
			Crustacea	-35	-20	-5
			Fish	-10	-10	-30
		July 2017	Algae	-29	-48	21
			Bacteria	57	45	60
			Crustacea	-15	-5	-15
			Fish	-30	-20	-20
	16962	October 2016	Algae	-81	18	-81
			Bacteria	21	-56	21
			Crustacea	0	-70	0
			Fish	-60	0	-60
		January 2017	Algae	-83	-46	-66
			Bacteria	-24	-25	-19
			Crustacea	-40	-55	-45
			Fish	-30	-10	-30
		April 2017	Algae	-11	-84	18
			Bacteria	-5	-5	-7
			Crustacea	-40	-5	-20
			Fish	-40	-20	0
		July 2017	Algae	26	-36	-16
			Bacteria	50	36	50
			Crustacea	-15	-50	-10
			Fish	-50	-70	-40
	27186	October 2016	Algae	-38	99	-26
			Bacteria	14	28	5

Sector	Site	Date	Test	Inhibition (-)/stimulation (+) Upstream	Effluent	Downstream
Mining	27186	October 2016	Crustacea	0	-50	0
			Fish	-40	-10	-10
		January 2017	Algae	-65	-4	-6
			Bacteria	-50	-7	-26
			Crustacea	-35	-10	-35
			Fish	0	-30	-20
		April 2017	Algae	19	-70	-5
			Bacteria	-9	-38	-4
			Crustacea	-10	-5	-20
			Fish	-30	-20	-20
		July 2017	Algae	-38	-34	9
			Bacteria	68	18	44
			Crustacea	0	-30	-10
			Fish	-30	-100	-50
	91444	October 2016	Algae	-82	45	8
			Bacteria	28	29	26
			Crustacea	-25	-25	-50
			Fish	0	0	0
		January 2017	Algae	-68	5	-33
			Bacteria	-19	-27	-24
			Crustacea	-35	-35	-35
			Fish	0	-30	-10
		April 2017	Algae	-38	-33	-3
			Bacteria	-3	-4	-2
			Crustacea	-25	-20	-55
			Fish	-10	-10	-20
		July 2017	Algae	21	-1	-4
			Bacteria	88	53	57
			Crustacea	0	-25	-10
			Fish	-40	-40	-20
Municipal	43114	September 2016	Algae	21	-100	21
			Bacteria	22	-100	22
			Crustacea	-5	-100	-5
		November 2016	Algae	81	-101	-45
			Bacteria	-18	-100	-25
			Crustacea	-40	-100	-30
		January 2017	Algae	20	-48	-25
			Bacteria	-24	-15	-20
			Crustacea	-30	0	-70
		January 2017	Fish	-30	-20	-30
			Algae	13	-96	0
			Bacteria	-11	-40	-6
			Crustacea	-20	-10	-15

				Inhibition (-)/stimulation (+)		
Sector	**Site**	**Date**	**Test**	**Upstream**	**Effluent**	**Downstream**
Municipal	43114	May 2017	Algae	-19	57	25
			Bacteria	-15	-25	-8
			Crustacea	-20	0	-20
		July 2017	Algae	-56	-102	-76
			Bacteria	-20	-100	-20
			Crustacea	0	-100	-10
	49909	September 2016	Algae	-8	-101	-9
			Bacteria	29	-100	29
			Crustacea	0	-100	-20
		November 2016	Algae	-28	9	-75
			Bacteria	-21	-6	-19
			Crustacea	-15	-100	-35
		January 2017	Algae	72	27	63
			Bacteria	-7	-6	-33
			Crustacea	-30	0	-30
			Fish	-40	-10	-40
		March 2017	Algae	59	3	59
			Bacteria	-11	-35	-11
			Crustacea	-15	-25	-25
		May 2017	Algae	-52	-71	31
			Bacteria	-13	-20	-3
			Crustacea	0	-100	-20
		July 2017	Algae	-4	70	23
			Bacteria	-38	52	-42
			Crustacea	0	-100	-100
	64033	September 2016	Algae	37	-42	40
			Bacteria	20	5	23
			Crustacea	-5	-45	0
		November 2016	Algae	7	-32	29
			Bacteria	-23	-100	-21
			Crustacea	-25	-35	-30
		January 2017	Algae	64	-82	10
			Bacteria	-10	-84	-16
			Crustacea	-35	-25	-5
			Fish	-50	-20	-40
		March 2017	Algae	46	-100	67
			Bacteria	-12	-100	-22
			Crustacea	-35	-100	-25
		May 2017	Algae	-20	-111	-94
			Bacteria	-14	-100	-73
			Crustacea	-45	-100	-100
		July 2017	Algae	79	-100	-101
			Bacteria	-46	-100	-90

Sector	Site	Date	Test	Inhibition (-)/stimulation (+) Upstream	Effluent	Downstream
Municipal	64033	July 2017	Crustacea	-5	-100	-100
	71056	September 2016	Algae	-13	-99	-11
			Bacteria	31	-31	24
			Crustacea	-20	-100	-20
		November 2016	Algae	-16	30	-17
			Bacteria	-32	-44	-36
			Crustacea	-30	0	-30
		January 2017	Algae	36	9	54
			Bacteria	-14	-3	-19
			Crustacea	-60	-15	-30
			Fish	-50	-20	-40
		March 2017	Algae	73	-18	60
			Bacteria	-9	-39	-10
			Crustacea	-5	0	-15
		May 2017	Algae	45	-101	32
			Bacteria	-37	-61	-35
			Crustacea	-15	-100	-25
		July 2017	Algae	64	29	64
			Bacteria	-21	45	-21
			Crustacea	-10	-5	-5
	75130	September 2016	Algae	44	-100	-22
			Bacteria	23	-100	31
			Crustacea	-5	-100	0
		November 2016	Algae	-16	59	-1
			Bacteria	-21	-25	-20
			Crustacea	-30	0	-15
		January 2017	Algae	72	-104	76
			Bacteria	-19	-87	-22
			Crustacea	-30	-100	-65
			Fish	0	-100	-40
		March 2017	Algae	54	-101	79
			Bacteria	-15	-28	-31
			Crustacea	-10	-100	0
		May 2017	Algae	-5	-98	-96
			Bacteria	-10	0	-41
			Crustacea	-20	0	0
		July 2017	Algae	57	-102	27
			Bacteria	-20	-100	-23
			Crustacea	0	-100	0