Implementation of Ecological Hazard Assessment of Industrial Waste Discharge: A Comparison of Toxicity Test Methods

Report to the
WATER RESEARCH COMMISSION

by

NJ Griffin, WJ Muller, AK Gordon
Unilever Centre for Environmental Water Quality
Institute for Water Research
Rhodes University

WRC Report No. KV 276/11
ISBN 978-1-4312-0156-3

September 2011

The publication of this report emanates from a project entitled *Implementation of Ecological Hazard Assessment of Industrial Waste Discharge: a comparison of toxicity test methods* (WRC Project No. K8/748).

EXECUTIVE SUMMARY

BACKGROUND

The National Water Act (No. 36 of 1998) provides for water in sufficient quantity and in sufficient quality for basic human needs and for maintenance of aquatic ecosystem function. In order to achieve this, discharge of effluents into water bodies needs to be managed so as not to compromise water quality. In the past, the main method for assessing whether effluents could be discharged was based on discharge criteria structured around substance-specific guidelines. Although useful in management of effluent discharge, these were found to have flaws as a result of the difficulty in predicting toxicity owing to interactions between individual compounds and each other as well as the environment, an incomplete understanding of their toxic effects, and the massive number of potential toxicants and the cost and difficulty of analysing all of them. An approach known as Direct Estimation of Ecological Effect Potential (DEEEP) was proposed as a means of circumventing the shortcomings of direct toxicant monitoring. DEEEP proposes a battery of tests to directly assess effluent oxygen demand, lethal (acute) and sublethal (chronic) toxicity, bioaccumulation, mutagenicity and persistence potential of effluents, using test organisms from a range of trophic levels. DEEEP is currently being phased in to utilize the experience and skills available in the country, to test and refine the methods, and to allow for the creation of systems for training and skills transfer and for information management.

RATIONALE

When proposed, it was recognised that implementation of widespread toxicological testing in South Africa may be limited by the base of skills and facilities for toxicological testing. One possible route to simplify adoption of testing may be the use of commercial toxicity test kits that can be used to undertake many of the tests proposed under DEEEP. The kits require relatively little labour to undertake and remove the necessity of maintaining cultures of test organisms. This project examines the suitability of commercial toxicity test kits for use under DEEEP in South Africa.

The assays proposed under DEEEP are all widely used and widely tested internationally. A criticism may be raised that these assays, relying on standard test organisms, may have little ecological relevance in South Africa. This project addresses this question and examines a number of assays using indigenous test taxa in comparison with the standard tests proposed under DEEEP.

OBJECTIVES AND AIMS

- The different assays under consideration will be compared on the basis of a literature review assessing endpoint defensibility and the ecological relevance of tests.
- The sensitivities of the different assays under consideration will be compared by testing four complex effluents. Three of these derive from a range of industries, and the fourth is wastewater treatment plant effluent.

- All toxicological assays undertaken will undergo a complete cost analysis, and the results will be compared with the costs of outsourcing tests to external laboratories.
- The suitability of the assays under consideration will be compared on the basis of sensitivity, cost and ease of implementation for use in routine DEEEP testing, particular at smaller or municipal laboratories.

DEFENSIBILITY OF ENDPOINTS AND ECOLOGICAL RELEVANCE

Endpoints that are defensible are those from bioassays that produce repeatable, reproducible and comparable results. Many standard assays have been widely tested and standardized. Where toxicity test kits are based on standardized methods, the results generally concur. Non-standardized tests, including those using native taxa, need further assessment to determine the defensibility of their endpoints.

For true ecological relevance, an assay should be undertaken on a community representative of that into which an effluent is to be discharged. Such tests can be difficult and costly to undertake. If single-taxon assays are to be used, the test organism should be a dominant or keystone one from the area that effluent is to be discharged.

Approximately half of the tests assessed in this study follow standardised methods, or use kits that are based on standardised tests, and these should produce results that are defensible. In addition, although no tests assessed in this study assess impacts at a community level as all are single-taxon tests, the majority of tests have ecological relevance in that the test taxa have been reported from South African waters.

COMPARISON OF TEST RESULTS USING FOUR COMPLEX EFFLUENTS

Endpoints generated by all assays assessed in this report were compared after determining the toxicity of four complex effluents. Test taxa included laboratory-reared and wild-collected organisms, as well as hatched organisms from selected commercial toxicity test kits. Complex effluents used in tests were collected from a municipal wastewater treatment works, a tannery, a dairy and a textile plant, and were stored at −18°C before use. Selected physico-chemical parameters were also collected for each effluent. Endpoints derived were all either LC_{50} or EC_{50} values.

Many assays did not produce valid endpoints as a result of excessive mortality or inhibition at the lowest effluent concentrations tested, or insufficient mortality or inhibition at the highest effluent concentrations tested, or growth stimulation relative to controls. In cases where repetition of the assay over an extended range of dilutions might return a result, assays were repeated if sufficient stored effluent was available for retesting. Of the effluents tested, tannery effluent was most frequently associated with excess mortality or inhibition of test taxa. Tests using algae as test taxa generally showed growth stimulation in effluents from a municipal wastewater treatment works and a dairy.

Direct comparison of endpoints from standard tests based on cultured test organisms with those of matching tests that use commercial test kits was complicated by the failure of initial tests to return precise endpoints. Underlying causes for this varied, though excess mortality or inhibition at the lowest effluent levels tested, growth stimulation on exposure to effluent,

and interference of coloured effluent in optical density readings all contributed to difficulties encountered in endpoint derivation.

The *Caridina nilotica* juvenile lethality test returned endpoints for three of the four effluents assessed, mostly without needing retesting using a modified dilution series. As a result, this test ranks as the most tractable of the tests assessed here. In addition, this test proved more sensitive than others for two of three effluents when compared with other tests that returned endpoints. As *C. nilotica* is a South African native, this test deserves consideration for use on the basis of effectiveness, sensitivity, and ecological relevance.

After the *C. nilotica* juvenile lethality test, the test that returned the next highest number of endpoints was the *Vibrio fischeri* bioluminescence test. This test was among the least sensitive of the assays assessed.

Two of the effluents assessed during this study had distinct colour casts. Coloured or opaque effluents interfere in assays where assessments of toxicity rely on measures of optical density or luminescence, or counts of especially smaller taxa where individuals might more easily be overlooked. It would be more appropriate in such cases to use a test where effluent colour or opacity does not affect the assay outcome. Assays using *Lemna* spp as test taxa have been recommended elsewhere as appropriate for such effluents.

Problems encountered in obtaining endpoints without retesting of samples in this study arose in a fully equipped research laboratory staffed with experienced researchers. As such it is apparent that many sources of potential error and difficulty exist that will complicate instituting routine toxicological testing in South Africa. It is therefore of considerable importance that methodologies for routine testing are refined and developed and that such methodologies should include specific steps to address problems that may arise at any phase of testing.

An international trend exists for the use of kits in toxicological testing so as to reduce the load and cost of maintaining cultures of test organisms. Adoption of kits, once adequately tested, for use in South Africa may facilitate instituting routine toxicological testing under DEEEP and the National Toxicity Monitoring Programme (NTMP). However, owing to difficulties obtaining endpoints for assays assessed in this study, further testing is recommended before unreserved adoption of these kits for use in toxicological testing.

COMPARISON OF COST OF TOXICOLOGICAL TESTING

When a toxicological test has ecological relevance and when it generates reproducible and defensible endpoints, selection of the test will be influenced by test costs. Test costs are determined by the costs of equipment and consumables for testing as well as time and skill requirements for assay completion. Where cultures are maintained for the production of test organisms, the costs of culture maintenance add to the overall cost per test. Costs of culture maintenance have been identified internationally as contributing significantly to costs of culture-based tests, and this underlies the development of kits that can be used to undertake assays without maintaining cultures of test organisms.

The costs of undertaking toxicological tests using kits, standard culture-based tests, and tests using indigenous organisms were compared, looking at overall costs of equipping a

laboratory for testing, costs of undertaking tests, and costs, where appropriate, of maintaining cultures for tests. Potential savings owing to sharing of equipment between assays and to re-use of equipment when undertaking multiple assays were also considered. Finally, costs of testing were compared with the costs of outsourcing testing to external laboratories.

Of the outsourced tests, the cheapest DEEEP tests were the chemical (COD) and biological oxygen demand (BOD) tests. The cheapest of the toxicological tests was the *V. fischeri* acute test, and costs per test increased gradually through the *Daphnia pulex* lethality test to the fish (*Poecilia reticulata*) lethality test and then to the algal (*Selenastrum capricornutum*) growth inhibition test. The *D. pulex* reproduction test was significantly more expensive than the other toxicological tests.

The costs of equipping a laboratory for undertaking the assays assessed here varied widely. The most expensive equipment requirements were those of the algal growth inhibition tests, followed by the *D. pulex* reproduction test and the *D. pulex* lethality test, all using cultured test taxa. Following these are costs for equipping a laboratory to test using commercial toxicity test kits, with the equipment for using the Biotox kit at most expensive, followed by that for the Protoxkit F, and then the Algaltoxkit F. Equipment requirements for the remaining kits are substantially cheaper and equipment for the remaining tests using laboratory-reared and wild-collected indigenous test taxa cheaper still.

Costs per test comprise of costs of consumables used during testing, labour costs, and where applicable, costs of producing appropriate test organisms in cultures. In terms of labour and consumable costs, the mayfly 10 day lethality test is the most expensive of all tests assessed. The *D. pulex* reproduction test is a close second, and, once the cost of culturing test organisms for the test is factored in, is overall the most expensive of the tests assessed. The *C. nilotica* juvenile lethality test, *C. nilotica* 10 day lethality test and the algal growth inhibition test using the Algaltoxkit F kit cost slightly more than half the cost of the most expensive tests when all costs are combined. In the tests using *C. nilotica*, culture costs make up more than half of the overall test cost. The majority of the remainder of the tests assessed here cost between R500 and R1 000 per test with all costs included. The cheapest of the tests assessed was the *D. pulex* lethality test using the Daphtoxkit F pulex. The next cheapest test was the *V. fischeri* bioluminescence test using the Biotox kit, followed by any of the algal growth inhibition tests that used laboratory cultured algae as test taxa. In general, costs of culture-based tests reflect the large amount of time (as labour costs) required for testing and in particular for culturing of test organisms. The costs of toxicity testing using commercial toxicity tests kits largely comprise the cost of the kit in question, as labour costs are relatively low.

When the equipment requirements of the various tests assessed are reviewed, it becomes apparent that high equipment costs are frequently a consequence of one or a few particularly expensive items. Where these items are not dedicated to the test in question but may be used for more than one kind of test, the overall equipment costs of both tests may be reduced. In an attempt to determine the potential cost savings due to sharing of equipment between tests, various different scenarios were investigated.

The results indicate that considerable savings can be realised by adding a new test to a suite of tests already present, to the extent that, in many cases, new equipment requirements of added tests could be reduced to zero. This was particularly the case when a new test was added to a test suite that already used the test taxon to be added, as tests using the same test taxon frequently shared much of their equipment. Another reason for large savings on equipment was a function of tests sharing a requirement for a single expensive equipment item (e.g. water purifier, incubator). Where equipment costs of adding a test are reduced to zero or near zero, the cost per test of the added test is reduced to the cost of labour and consumables for testing, and where applicable, test organism culture.

Equipment costs, when sharing between tests is not possible, may make up a large proportion of the cost of introducing a test to a laboratory. When equipment items can be frequently re-used, the cost of the item, expressed on a per test basis, may be reduced. Of all the tests assessed, the algal growth inhibition test using cultured algae showed the greatest potential for cost reduction on the basis of equipment re-use. This is a function of the high equipment requirements of this test combined with a low cost of individual tests. In contrast, if few test repetitions are planned, the mayfly 10 day lethality test, with its low equipment requirements, is among the cheapest of tests. After many repetitions, when the price per test is strongly affected by the test rather than equipment costs, the mayfly 10 day lethality test is among the most expensive of assays assessed. While no clear pattern is visible after few test repetitions, after tests have been repeated 100 times or more, tests using commercial test kits tend generally to be cheaper than their counterparts using cultured or wild-collected test organisms. In many cases, this is due to the high ongoing cost of culturing organisms for use in tests.

The costs of culture maintenance for testing were derived on the assumption that tests were undertaken at the maximum rate supported by the culture facilities. Should the testing rate be lowered, the cost on a per test basis of culture maintenance would increase. For this reason it is important that culture facilities should be appropriately sized when producing organisms for toxicological testing. Should testing be slower than the maximum rate supported by the culture facilities, or if testing is infrequent or irregular, the cost of test organism production will increase the cost of testing. In such cases, the use of commercial test kits that do not rely on culture facilities at the test site may prove more cost effective.

The number of tests repetitions envisaged is an important factor in considering which test would be more cost effective. Should few repetitions be required, outsourcing of testing will be cheaper. Once equipping a laboratory and undertaking the test on-site reaches cost parity with outsourcing, users will have to look at specific test costs and comparisons between different methods before choosing a solution most appropriate to their circumstances.

DISCUSSION

Outsourcing of testing offers an opportunity to undertake assays in an accredited laboratory with trained staff. However, unless only a few tests are required, outsourcing of testing will generally not be the most cost effective solution.

Direct comparison of the standard culture-based and commercial kit-based assays mandated under DEEEP revealed no obvious differences in endpoints between each pair of

tests. However, better results are needed to conclusively state that the same assay, undertaken using kit-based and culture-based methods, gives the same endpoints. The *D. pulex* lethality test undertaken using standard methods and a commercial kit had comparable cost only if the cost of culturing test organisms was not considered. However, costs were similar enough that equipment choice could affect whether one test or the other proved more cost effective. The *Pseudokirchneriella subcapitata* growth inhibition test is substantially cheaper when undertaken following the standard method rather than using the commercial kit, but equipping a laboratory for the standard test is expensive. As a result, the standard method is more cost-effective only when a large number of tests are undertaken. The *V. fischeri* bioluminescence test was only represented in this study by the Biotox kit. This test proved cost-effective and tractable, though one of the least sensitive of those assessed here.

The *C. nilotica* juvenile lethality test proved to be tractable and sensitive compared to other tests assessed here. Although the cost of undertaking the test is low, the cost of producing test organisms is high. Together these costs make this test one of the more expensive of the tests assessed. Despite the high overall cost of this test, its sensitivity and ecological relevance recommend this method for consideration for regular application in South Africa. Results from this study are insufficient to assess the potential of the *C. nilotica* 10 day lethality test and further research in this regard is recommended.

Application of the mayfly 10 day lethality test was complicated by the very large quantities of effluent required for testing, as these precluded re-testing of samples and raised issues relating to the disposal of large quantities of toxic effluent. Although the test has ecological relevance it is one of the more labour-intensive and therefore costly tests. Overall, further research is needed to determine the usefulness of this test, as well as to ascertain endpoint variability owing to taxonomic variation among the mayflies used as test taxa.

Use of any of the culture-based algal growth inhibition tests was complicated by colour casts in two of the four test effluents that precluded accurate spectrophotometric assessment of algal growth. All algal growth inhibition tests with indigenous algae had some ecological relevance. The tests were relatively low-cost (including production of test organisms); however, the cost of equipping a laboratory for these tests and associated cultures was high. Owing to a combination of growth stimulation in certain effluents, and difficulty in spectrophotometric readings in coloured effluents, it is not possible to reliably comment on the differing sensitivities of algal taxa assessed here.

The *Brachionus calyciflorus* reproduction inhibition test, as undertaken using the Rotoxkit F test kit, is one of two of the tests assessed here that measure changes in reproduction of test taxa. Preliminary results suggest that the test is sensitive, though further research is needed to confirm this. As *B. calyciflorus* is native to South Africa the test has ecological relevance. Together with a moderate cost per test, these results suggest that this test and test kit deserve further consideration for routine application in South Africa.

The remaining tests that were assessed all used test kits, and, for various reasons including low ecological relevance, cost and tractability, these tests do not show much promise for routine application in South Africa.

CONCLUSIONS

The research presented here highlights a range of issues related to routine toxicological testing of whole effluent samples. In particular, methods for approaching testing of coloured or opaque effluents need to be included in the DEEEP methodology. In addition, methods for application when effluents are either mildly toxic or very toxic, when endpoints might not be revealed using standard methods, need to be elucidated to avoid users working to non-standardized systems.

Several assays assessed here seem worthy of further investigation for routine application in South Africa on the bases of ecological relevance, ease of use, and, in most cases, cost-effectiveness. These tests are outlined above.

Careful planning allows laboratories to rationalise test selection in order to be capable of undertaking several tests at relatively low cost. Planning for test selection facilitates undertaking tests at reasonable cost as well as adding tests to a suite of others already undertaken. Decisions regarding test selection will depend on the goals of the laboratory in question; however, it is clear that planning will facilitate balancing tests offered, test throughput and cost of testing.

When culture facilities are required in a laboratory, that facility may enable further tests to be undertaken using the same test taxon without requiring further capital equipment purchases, as tests that share test taxa often share equipment too.

As a general rule, toxicological testing using kits may be undertaken at lesser overall cost than toxicological testing using test organisms from cultures. The reason for this lies in the high cost of maintaining cultures for production of test taxa. Cost of cultures varies considerably between different taxa. In some cases, costs of culturing test taxa may exceed the cost of undertaking tests using that organism. Not all test taxa can be immobilized for distribution with kits, however, and where whole organism tests are required, it may be necessary to maintain cultures for production of test taxa.

RECOMMENDATIONS FOR FUTURE RESEARCH

DEEEP at present contains no method specifically for use with coloured or opaque effluents. This is a serious oversight, as a number of DEEEP-mandated assays are not easily undertaken with coloured effluent. It is suggested that a test be included in methodological manuals that can be used when light penetration through effluent is reduced. This would fill a similar role as the deployment of tests using e.g. *Lemna* spp elsewhere in the world.

Methodological manuals for DEEEP tests need to include specific steps to follow when testing fails for various reasons. One reason is that effluents can prove too toxic for a set dilution range to return an accurate effect value. Retesting using the same effluent will often not be possible owing to limited retention time of samples under DEEEP, and alternatives need to be explicitly laid out. Another possible reason for not obtaining effect values arises when effluents are only mildly toxic and certain endpoints may not be calculable. This may be addressed by the selection of alternate endpoints or by classifying endpoints of effluents in ranges derived following consideration of the Resource Quality Objectives (RQO) for the receiving waters.

CAPACITY BUILDING

This project offered an opportunity to develop scientific skills in several students and early career water scientists at the Institute for Water Research at Rhodes University. Ms NP Gola undertook the work on algal growth inhibition tests, the development of which is part of her PhD. Mr AR Slaughter worked on the project while completing his PhD, and Ms N Ketse, then a research intern, was also engaged in toxicological testing for this project.

ACKNOWLEDGEMENTS

The authors would like to thank the following people and organisations for input to and help with this project:

The Water Research Commission for funding the research presented here. In particular, we would like to thank Annatjie Moolman, Jan du Plessis, Chris Moseki and Kevin Murray for input and support.

Hesmarie Pearson and Christian Loots of Toxsolutions Kits and Services who supplied the toxicity test kits used during this project, as well as undertaking all testing using the kits and providing a breakdown of equipment, consumable and labour costs involved in testing with the kits.

Members of the Unilever Centre for Environmental Water Quality for support in collection of effluents and mayflies and for undertaking the toxicological test using wild-collected or laboratory-cultured test organisms. Pearl Gola, Nosi Ketse, Andrew Slaughter and Tarqyn Human all contributed throughout the project. Roman Tandlich assisted with chemical and biochemical testing of effluents. Alexandra Holland contributed to the costing of the tests, and Juanita McLean and David Forsythe offered administrative support.

The laboratories of Rand Water, Golder Associates (Pty) Ltd, Renaissance Environmental Hub, the East Rand Water Care Company (ERWAT) and the CSIR for help with costing of outsourcing of testing.

Dr Sanet van Vuuren of North-West University for her input on the use of algae for testing and on the distributions of the algae used in toxicological testing.

Several anonymous organisations and companies who afforded us an opportunity to collect effluent intended for release for use in testing.

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LIST OF ABBREVIATIONS

DEEEP	Direct Estimation of Ecological Effect Potential
DTA	Direct Toxicity Assessment
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
ISO	International Organization for Standardization
IWR	Institute for Water Research
NRA	National Rivers Authority
NTMP	National Toxicity Monitoring Programme
RDM	Resource Directed Measure
RQO	Resource Quality Objective
RSA	Republic of South Africa
SDC	Source Directed Control
TEM	Totale Effluent Milleuhygiene
UCEWQ	Unilever Centre for Environmental Water Quality
UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
WET	Whole Effluent Toxicity
WWTW	Wastewater Treatment Works

1 INTRODUCTION

1.1 Background

The National Water Act (No. 36 of 1998) of South Africa provides for water of sufficient quantity and quality to sustainably maintain and use aquatic ecosystems, as well as for basic human needs (RSA, 1998). The discharge of waste into aquatic systems is permitted where no other viable alternative for disposal exists and where the waste discharged poses a sufficiently small hazard to the environment. Management of waste discharged in this way has in the past been largely based on discharge criteria which are structured around substance-specific guidelines (Jooste and Herbst, 2004).

The application of discharge criteria based on a waste material's chemical composition alone has a number of drawbacks (e.g. see DWAF, 2003). Broadly, these relate to the difficulty in predicting toxicity owing to interactions between individual compounds and each other as well as the environment, an incomplete understanding of their toxic effects, and the massive number of potential toxicants and the cost and difficulty of analysing all of them. Whole effluent toxicity testing offers a way of directly assessing the effect of the effluent on biological systems without prior knowledge of the chemical constituents of the effluent. The then Department of Water Affairs and Forestry (DWAF) proposed introducing whole effluent toxicity testing as a means of providing an integrated assessment of the potential effects of waste effluent discharge, and entitled this the Direct Estimation of Ecological Effect Potential (DEEEP) approach (DWAF, 2003). DEEEP was based on an assessment of programmes applied in other countries, with adaptations 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).

The implementation of DEEEP was to be phased so as to make use of the experience and skills that exist in the country, to test and refine the methods, and to allow for the creation of systems for training and skills transfer and for information management (Jooste and Herbst, 2004). This implementation was not complete at the time of writing. One limitation to full implementation of DEEEP was identified as the limitation of human resource and infrastructural capacity for undertaking the assays in South Africa (Jooste and Slabbert, 2006). Many of the assays mandated under DEEEP can be undertaken using commercial toxicity test kits that simplify testing and remove the requirement for maintenance of cultures of test organisms. As such, these kits may partially address limits on testing imposed by lack of capacity. Several authors have compared the results of the same test undertaken using toxicity test kits and standard methods and, though there are exceptions, the consensus seems to be that no significant differences exist in the endpoints from these methods (Blaise, 2000; Janssen et al., 2000; Mitchell et al., 2002; Daniel et al., 2004; Persoone et al., 2009; Persoone and Wadhia, 2009). As commercial kits that undertake many of the DEEEP assays are available, these could potentially increase capacity for toxicological testing throughout the country.

The tests selected for DEEEP are all standard tests that are widely used and recognised internationally. While the use of standardized tests lends itself to effective law enforcement (DWAF, 2003), it has been argued that toxicological testing needs to consider the impact of potential toxicants on the ecosystems that would be exposed to them rather than on a small

number of standard test organisms (Chapman, 2002; Preston 2002). In this light, it is of value to compare the endpoints derived from assays undertaken using DEEEP methods with tests on native or indigenous taxa. Such a comparison would help elucidate whether the assays selected for DEEEP have ecological relevance to conditions in South Africa for which they are employed.

1.2 Objectives

This report aims to assess assays used in DEEEP in comparison with assays undertaken using commercial toxicity test kits and in comparison with toxicity tests using cultured or wild-collected native taxa as test organisms. Comparisons will be made on the following bases:

- The different assays under consideration will be compared on the basis of a literature review assessing endpoint defensibility and ecological relevance of tests.
- The sensitivities of the different assays under consideration will be compared by testing four complex effluents. Three of these derived from a range of industries, and the fourth will be wastewater treatment plant effluent.
- All toxicological assays undertaken will undergo a complete cost analysis, and the results will be compared with the costs of outsourcing tests to external laboratories.
- The suitability of the assays under consideration will be compared on the basis of sensitivity, cost and ease of implementation for use in routine DEEEP testing, particularly at smaller or municipal laboratories.

2 IMPLEMENTATION OF ECOLOGICAL HAZARD ASSESSMENT OF INDUSTRIAL WASTE DISCHARGE AND A COMPARISON OF TOXICITY ASSAYS.

2.1 The need for a bio-assessment approach when determining the ecological hazard of waste discharge.

According to the National Water Act, before the discharge of wastewater is allowed, the effect of the wastewater on the environment must be assessed (RSA, 1998). Such assessments have usually relied on water quality guidelines or waste discharge standards, which measure the hazard of a single substance or chemicals in the wastewater (DWAF, 2003). However, there is growing realization that this approach is limited and a different approach is needed to supplement these substance specific guidelines. The limitations of substance specific hazard assessment is detailed in DWAF (2003), and summarized below:

- In order to utilize substance-specific guidelines the composition of the wastewater must be known. However, not all components might be known, and furthermore, of those substances known, only relatively few have been characterized toxicologically. These "few" might still amount to some thousands, making the assessment of a wastewater sample uneconomical.
- As a consequence of synergistic and antagonistic behaviour of different single chemicals in a mixture, a complex wastewater mixture may have a different toxicological effect compared to the single chemicals making up its composition. Furthermore, the toxicity of wastewater mixtures can change over time, making assessment of the toxic components often impossible.
- Some chemical substances can cause significant toxicity at levels that are below detection limits, making the substance specific approach redundant.

In order to address and attempt to mitigate the above limitations, many countries are using bio-assessment techniques to assess the ecological hazard of wastewaters. These bio-assessment techniques involve exposing organisms directly to the wastewater mixture and measuring a specific biological response. The advantages of this hazard assessment approach, called whole effluent toxicity (WET) assessment in the USA (USEPA, 1995) or direct toxicity assessment (DTA) in the UK (NRA, 1994), are that individual chemicals within the wastewater do not have to be identified, thus reducing costs, ensuring that synergistic and antagonistic relationships between chemicals are accounted for, and that any inability to detect dangerous chemicals within the wastewater is not a serious issue (DWAF, 2003).

As a result, the Department of Water Affairs (DWA) is developing a method for directly measuring the potential effect, or toxicity, or ecological hazard of complex wastewater effluents. Called Direct Estimation of Ecological Effect Potential (DEEEP), this method is intended to supplement the substance-specific assessments that are being utilized at present. The aim is to implement the method in a step-by-step fashion over time (DWAF, 2003) in order to allow for the technical development of the various tests, for the development of capacity needed to undertake these tests within South Africa, and for the gradual integration of the method into the current water resource regulation framework.

2.2 How does DEEEP fit into the water resource regulatory framework within South Africa?

The National Water Act (Act 36 of 1998) mandates the Department of Water Affairs (then the Department of Water Affairs and Forestry, or DWAF) to manage South Africa's water resources in a sustainable manner (RSA, 1998). In other words, the protection of water resources must be balanced with their development and use. Two primary approaches have been chosen to effect resource protection: Resource Directed Measures (RDMs) and Source Directed Controls (SDCs). Resource Directed Measures provide descriptive and quantitative goals for the desired ecological state of a water resource (these goals are termed Resource Quality Objectives or RQOs), while SDCs specify the criteria for controlling the impacts of water use (Palmer et al., 2004). As discussed in DWAF (2003), Section 21 of the National Water Act defines eleven water uses, one of which is discharge itself. Water use is managed by means of General Authorizations (when the water use has low potential of unacceptable impacts on the aquatic environment) and Licensed Uses (for those uses that, if not controlled, would have a high potential of unacceptable effect). The regulations make a distinction between general wastewater and complex wastewater in that the latter is arbitrarily assumed to contain 10% or more of industrial water (DWAF, 2003). The discharge of complex industrial wastewater is not generally authorized and as such users need to apply for a license. Licenses are subject to conditions including the specification of the quality of the wastewater being discharged in order to maintain the ecological goal set for the resource (RQO). It is in the specification of the license requirements that the primary application of DEEEP lies (DWAF 2003). However, DEEEP is not intended to replace the substance-specific approach used in water quality guidelines or general and special standards for end-of-pipe assessments, but rather complement these approaches (Jooste and Herbst 2004). This is necessary as, in order to manage the resource, resource managers still require chemical and physical criteria. They are not able to manage an effect of an effluent, without knowing which chemical or physical criterion needs addressing and to what extent (Jooste and Herbst 2004). During full implementation, the results of the DEEEP methodology should be clearly linked to and integrated into other aspects of the regulatory process, such as the development of RQOs and river health monitoring (Jooste and Herbst 2004). However, at present the primary application of DEEEP is in the authorization and control of complex industrial wastewater discharges. The obvious challenge at present is how to link DEEEP methodology (i.e. the derivation of toxicity units from various bioassays) with the goals set for the receiving water (i.e. RQOs, set either as substance-specific criteria, or as biomonitoring targets).

2.3 What does the DEEEP methodology comprise of?

Typically, effects-based assessments, like WET in the USA and DTA in UK, use a number of lethal (acute) and sublethal (chronic) bioassays to measure ecological hazard. The approach is flexible and a variety of tests and test organisms can be used, with countries selecting the most appropriate combination of tests to meet their particular requirements (Slabbert, 2004). The DWAF in South Africa found the Dutch TEM (Totale Effluent Milleuhygiene) approach the most suitable (Tonkes et al., 1999), as, in addition to lethal and sublethal toxicity tests, it also includes other hazard parameters such as oxygen depletion potential, bioaccumulation, and mutagenicity (Slabbert 2004). The hazard parameters included in the DEEEP approach are listed in Table 2.1.

What is a bioassay / toxicity test?

In the current context, a bioassay or toxicity test is an experimental procedure that measures, under defined conditions in the laboratory, the toxic effects of a mixture of chemical pollutants in an effluent on a group of living organisms or cellular system. The objective of undertaking toxicity tests is to attempt to predict the effect of the effluent on the aquatic ecosystem it is being released into.

Table 2.1 Ecological hazard parameters and tests that have, at present, been selected by DWAF for the DEEEP approach.

Parameter	Test
Oxygen demand	Chemical oxygen demand Biological oxygen demand
Short-term toxicity	Bacterium (*Vibrio fischeri*) Alga (*Pseudokirchneriella subcapitata)* Invertebrate (*Daphnia pulex*) Fish (*Poecilia reticula*)
Long-term toxicity	Invertebrate (*Daphnia pulex*)
Mutagenicity	Ames method – *Salmonella*
Bioaccumulation potential	HPLC estimation
Persistence	To be decided

This project aims to investigate endpoints generated for standard organisms when using commercially available toxicity test kits and standard tests using test taxa from laboratory cultures (see Table 2.2). The endpoints from these will then be compared with those generated from tests using indigenous or native taxa (both wild collected and laboratory raised). Comparison of the various tests will be according to the ecological relevance of endpoints generated, the defensibility of test endpoints, and the cost effectiveness of various toxicity test methods. The first two of these are explicitly considered below; the costs of the various tests are examined in depth in Chapter 4.

Table 2.2 Toxicity tests assessed in this study.

Standardised DEEEP tests	
	Daphnia pulex lethality test
	Daphnia pulex reproduction test
	Algal (*Pseudokirchneriella subcapitata*) growth inhibition test

Tests using cultured or wild-collected native taxa	
	Mayfly 10 day lethality test
	Algal (*Scenedesmus bicaudatus*) growth inhibition test
	Algal (*Chlorella* sp.) growth inhibition test
	Caridina nilotica juvenile lethality test
	Caridina nilotica 10 day lethality test
Commercial toxicity test kits	
	Algal *(P. subcapitata*) growth inhibition test (Algaltoxkit F™)
	D. magna lethality test (Daphtoxkit F™ magna)
	D. pulex lethality test (Daphtoxkit F™ pulex)
	Brachionus calyciflorus reproduction inhibition test (Rotoxkit F™ short-chronic)
	Tetrahymena thermophila growth inhibition test (Protoxkit F™)
	Vibrio fischeri bioluminescence test (BioTox kit)

What is an endpoint?

An endpoint can be described as the specific toxic effect measured in a bioassay or toxicity test – for example degree of mortality or lethality in a group of test organisms after 96h or 240h of exposure, or sublethal growth inhibition after 72h of exposure.

What does ecological relevance mean?

The objective of aquatic resource management is to protect aquatic ecosystem function. Ecological relevance refers to the extent to which the methods of bio-assessment (e.g. toxicity tests) and the end points they generate are likely to accurately predict or signal changes in the aquatic ecosystem. Thus, effects measured in a toxicity test exposing a population or community to an effluent are more likely to be ecologically relevant (i.e. predictive of ecosystem effect) than the effects measured from a number of unrelated single species. Furthermore, aquatic ecosystems typically experience long term exposure to chemicals at sublethal levels of toxicity. Consequently, more ecologically relevant toxicity tests are the ones with a longer duration of exposure relative to the life span of the organism, and also ones that measure sublethal endpoints such as growth inhibition and reproductive effects. Lastly, toxicity tests which use organisms that better represent the aquatic ecosystem in question (i.e. indigenous organisms) will be more ecologically relevant.

2.4 Ecological relevance of endpoints

A toxic response or endpoint can be measured at various levels of biological organization. The measurement of a toxic response at a natural population or community level has a high degree of ecological relevance, in that it is showing a direct ecological response to the

stressor. However, it is desirable to prevent ecological changes before they occur. One way to address this is by measuring stress responses at 'lower' levels of biological organization, such as the individual level (lethality endpoint), sub-organism level (sublethal endpoints measuring fertility or growth) and cellular and sub-cellular levels (physiological and biochemical responses) (Adams and Tremblay, 2003). At present it is not possible to confidently link effects measured at cellular and sub-cellular levels with changes at an ecosystem level. Consequently, water resource managers rely on changes in mortality (lethality) and growth or fertility (sublethality) as the earliest warning indicators of possible ecosystem effects. Although the effect of lethality as an ecological hazard is more obvious than growth or fertility, often only sublethal responses are observed in tests that measure lethal responses. Yet ecosystem effects can still occur when organisms are not killed outright by a stressor as that may affect the population to which that organism belongs through a reduction in fertility for example, leading to ecosystem changes. Consequently, both lethal and sublethal endpoints should be determined when assessing ecological hazard.

The longer a toxicity test the more ecologically relevant it is considered to be, as more of the organism's life cycle is exposed to the potential toxicant (Preston and Snell, 2001; Chapman, 2002). If the whole life cycle of an organism cannot be used in assays, or a combination of lethal and sublethal assays undertaken, then endpoints derived from toxicity tests exposing earlier stages of a test organism's life cycle are considered more relevant (Preston and Snell, 2001).

Some authors have indicated a difference in response of temperate and tropical test taxa to toxicants (Kwok et al., 2007), while other studies found that taxonomic affinity outweighed geographic and habitat differences (Maltby et al., 2005). In general though, one should be wary about extrapolating data from one geographic area to another (Chapman et al., 2006). For this reason, endpoints derived from organisms that are native to an area where an impact is anticipated or present are said to be more environmentally relevant as they are evolutionarily adapted to that aquatic environment. In this light, many standard test organisms are of northern hemisphere origin and not always adapted to conditions where they are used as test taxa.

As responses to toxicants of communities do not always mirror the responses of the individual taxa, even where these are known (Chapman et al., 1982), for true ecological relevance it has been proposed that toxicological assessments should undertake tests on mecocosms and/or on natural systems in the field (Chapman, 2002; Preston, 2002). Where assays are undertaken using single taxa, test taxa should be selected following surveys of site-specific ecological communities and should represent dominant or keystone taxa in local ecosystems (Chapman, 2002).

2.5 Defensibility of endpoints generated

The defensibility of the endpoints generated depends on whether the bioassay that generated the endpoints can provide reproducible, repeatable and comparable results, both within the same laboratory and at different laboratories. In addition, the endpoint should be clearly defined and should measure a biologically relevant toxic response (Tonkes et al., 2005). Many standardized laboratory organism bioassays and commercial toxicity test kits have been assessed to international standard (e.g. Römbke et al., 2009), while non-standardised tests using native species have not.

2.6 Assessment of toxicity test methods and their endpoints

The standard *D. pulex* tests used in DEEEP are based on widely recognised national and international standards and as such produce more defensible endpoints than the far less widely used tests using native taxa. Several of the commercial toxicity test kits assessed here are designed to follow national and international standards for standard tests, and as a result should also produce defensible endpoints. When tests using commercial kits are deployed, the test taxa for all kits derive from the same source and any variation that might occur between standard culture-based tests as a result of variation in the culture stocks is thereby avoided.

In general, direct comparisons between tests undertaken using toxicity test kits and the same tests using standard methods found no significant difference in the endpoints produced (Blaise, 2000; Janssen et al., 2000; Daniel et al., 2004; Persoone et al., 2009; Persoone and Wadhia, 2009). Exceptions have been found, however, and these are discussed in Mitchell et al. (2002). The latter authors note that the high throughput and low costs of toxicity test kits weigh in their favour. Blaise et al. (2000) conclude that replacement of standard tests with toxicity test kits is not unforeseeable.

In terms of ecological realism, native taxa should provide the most relevant endpoints, particularly if they are wild caught and to a lesser extent if they are laboratory reared. In addition the *Caridina nilotica* juvenile lethality test should be more environmentally relevant than most test methods as younger test taxa are used. The 21 day *D. pulex* reproduction test should also have greater ecological relevance owing to the length of exposure.

All taxa listed in Table 2.2 as native taxa were collected in South Africa. This improves the ecological relevance of the tests. *Daphnia pulex*, used as a standard test organism on many continents, has also been found in the country (Jarvis et al., 1987; Hart, 1992) (though populations may have been displaced by a *D. pulex* × *D. pulicaria* hybrid (Mergeay et al., 2006)). *Daphnia magna* has been reported from coastal lakes and pans in South Africa (Coetzer, 1981; Hart, 1995). *Brachionus calyciflorus* has also been reported from South Africa (Jarvis et al., 1987; Brain et al., 1995). No specific records of *P. subcapitata* from South Africa were found, although unidentified species from similar genera *Selenastrum* and *Kirchneriella* have been recorded (S Janse van Vuuren, pers. comm.) If the hotly disputed theory of cosmopolitan distribution of protists is accepted (e.g. see Finlay et al., 2002; Fenchel and Finlay, 2004), one might conclude that *P. subcapitata* would be found in South Africa provided that environmental conditions favoured its growth. Other authors however argue in favour of more limited distribution patterns and greater endemicity (Vyverman et al., 2007; Foissner et al., 2008). *Tetrahymena thermophila* has a limited distribution, and has not been found outside eastern North America (Foissner, 2006). *Vibrio fischeri* is a bioluminescent marine bacterium most commonly found in a symbiosis with various marine animals (Madigan and Martinko, 2006). As such, the ecological relevance of assays using the latter taxon is low.

3 COMPARISON OF TESTS: TOXICOLOGICAL TESTING USING STANDARD TEST ORGANISMS, INDIGENOUS ORGANISMS, AND COMMERCIAL TEST KITS

3.1 Introduction

The use of toxicity testing in managing environmental water quality and effluent quality is increasing world-wide (Grothe et al., 1996; Persoone et al., 2003) in order to improve the quality of effluent discharges and thus reduce or minimise risks and impacts to environmental waters. One of the principle motivations cited for this move is the uncertainty in accurately predicting risks to aquatic ecosystems, even following extensive physical and chemical analysis (Dewhurst et al., 2002; Manusadžianas et al., 2003; Wolska et al., 2007). Certainly, South Africa is attempting to follow this trend with the establishment of the National Toxicity Monitoring Programme (NTMP) (DWAF, 2005) and through the introduction of the Direct Estimation of Ecological Effects Potential (DEEEP) (DWAF, 2003), designed to monitor environmental water quality and effluent quality respectively.

One of the significant disadvantages to using living organisms in toxicity assessments of effluents and receiving or environmental waters has been identified as the effort and expense of maintaining cultures under laboratory conditions in order to have sufficient numbers of the selected species to undertake the toxicity tests (Persoone and Van de Vel, 1988; Persoone et al., 2003; Daniel et al., 2004). The development and use of commercially available toxicity testing kits sees a move away from the need for permanent cultures of selected test taxa requiring constant maintenance at some expense and usually by individuals with specialist expertise. These kits utilize the ability of some taxa to develop dormant or resistant stages which can be distributed with kits and hatched or reinvigorated prior to their use in toxicity tests. This circumvents the need for their maintenance in a laboratory culture, reducing laboratory running costs and removing one possible source of variability in test precision and accuracy as a result of subtle differences in both biotic and abiotic conditions of the laboratory culture. While the development of kits to circumvent this issue should go a long way towards increasing use of toxicity testing for management of environmental water quality and effluent quality, there are many questions that need to be addressed before they can be practically applied in all circumstances. However, despite the questions, the use of toxicity testing kits in aquatic toxicity testing has increased, as shown by an increase in publications in which these tests have been used (e.g. Blaise, 1998; Blaise 2000; Dewhurst et al., 2002; Mitchell et al., 2002; Manusadžianas et al., 2003; Daniel et al., 2004; Allan et al., 2006; Wadhia and Persoone, 2009).

There are several different methods available for toxicity testing within a regulatory framework, and both the NTMP and DEEEP have focused on a select few. However, in South Africa, the complexity of the proposed toxicity tests and the perceived costs associated with them have frequently been cited as key factors for their slow uptake in water resources management and effluent management. Some of these tests can be undertaken both by following standard methods using taxa from laboratory cultures as well as by using a commercial toxicity test kit. Differences in sensitivity between bioassays using laboratory cultured organisms and commercially available kits have been found (e.g. Persoone et al., 1994), however these may be a consequence of test taxon differences rather than differences between laboratory-cultured versus organisms hatched from the kits. In general though, most direct comparisons between standard assays undertaken using standard

culture-based methods and toxicity test kits found no significant difference between the endpoints returned (Blaise, 2000; Janssen et al., 2000; Daniel et al., 2004; Persoone et al., 2009; Persoone and Wadhia, 2009).

Although methods for toxicity testing exist that can be applied to a range of taxa (DWAF, 2000; Lahr et al., 2001), most tests use a relatively small range of standard test taxa, which commonly have a cosmopolitan distribution. Commercial toxicity test kits by their nature focus on standard test organisms. Although standard test taxa are used globally, it can be argued that these taxa are not always representative of local ecological communities and it may be better, in terms of realistically assessing potential impacts, to use indigenous taxa in toxicological testing (Chapman, 2002). A counter-argument to this is that use of a wide range of taxa complicates comparisons between test results.

In this chapter, results are presented for a suite of toxicity tests undertaken using laboratory-reared and wild-collected test organisms, and hatched organisms from selected commercial toxicity test kits. The results of tests using both standard test organisms and indigenous or native test taxa are also examined. Although the results of the toxicity tests in themselves fulfil one of the objectives of the project, the results and experiences gleaned from application of a suite of effluent toxicity tests are discussed, particularly in light of the practical implementation of effluent testing in environmental water quality management.

3.2 Methods and materials

Effluents were collected from four different representative sources in the Eastern Cape and subjected to a battery of selected toxicity tests using test organisms derived from laboratory cultures or collected from the wild, as well as dormant or immobilized material in commercial toxicity test kits. The effluents selected for testing were discharges from a wastewater treatment works (WWTW), a tannery, a dairy and a textile plant (Figure 3.1).

Figure 3.1 Samples of effluent tested showing colour cast.

When collecting effluent samples, sufficient effluent needed to be collected for all tests so as to have a single batch of each effluent to standardize effluent quality between tests and

thereby allow meaningful comparison of test results. Freezing of effluent samples is proscribed under DEEEP (Slabbert, 2004), and Davies-Coleman (2001) found freezing may modify the chemical properties of stored complex textile effluent. On the other hand, Latif et al. (1995) found that freezing had no effect on the toxicity of effluents to *Daphnia magna*. The alternative to freezing a single batch sample of effluent is repeated collection of fresh effluent prior to testing, which would introduce variation in test effluents as a result of temporal variation in discharged effluent. In the interests of avoiding temporal variation in effluent quality, 125 ℓ of each of the four effluents assessed during this study were collected together, before being frozen at -18ºC in containers containing an appropriate volume for each of the tests. When effluent was required for testing, the appropriate quantity of effluent was defrosted to room temperature.

In addition to the effluent samples required for toxicity testing, a small sample was also required for testing selected physico-chemical parameters. Standard protocols were followed for the chemical analyses (nitrate, nitrite, ammonia, ortho-phosphate, chemical oxygen demand and 5-day biological oxygen demand) while physical parameters were measured using relevant meters (pH, dissolved oxygen, electrical conductivity and temperature) at the time of sampling.

The same effluent dilution range was applied for toxicity tests using each of the effluents to ensure uniformity of testing between the different methods. The standard effluent concentrations tested were 100%, 50%, 25%, 12.5% and 6.25%, though, in some cases, other dilution ranges were used, in the interests of obtaining a valid LC_{50} or EC_{50} endpoint. LC_{50} or EC_{50} endpoints were selected for comparison as these are widely used and, as the slope of the dose-response curve is generally steepest at the midpoint, these endpoints should be more accurate and less variable (Mitchell, 2002);.

Table 3.1 A list of all toxicity tests undertaken as part of the project, including the source of the test organisms. Where commercial kits are used, kit manufacturers are given in parentheses.

	Test	**Source of test taxa**
Tests in NTMP and DEEEP	*Daphnia pulex* lethality test (48hr LC_{50})	Laboratory culture and Daphtoxkit F™ pulex kit (MicroBioTests Inc.)
	Daphnia pulex reproduction test (21 day EC_{50})	Laboratory culture
	Algal *(Pseudokirchneriella subcapitata*) growth inhibition test (72hr EC_{50})	Laboratory culture and Algaltoxkit F™ kit (MicroBioTests Inc.)
	Vibrio fischeri bioluminescence test (30 min EC_{50})	Biotox kit (Aboatox Oy)
Other tests	Mayfly 10 day lethality test (10 day LC_{50})	Field-collected Leptophlebiidae and Tricorythidae
	Daphnia magna lethality test (48hr LC_{50})	Daphtoxkit F™ magna kit (MicroBioTests Inc.)
	Tetrahymena thermophila growth inhibition test (24hr EC_{50})	Protoxkit F™ kit (MicroBioTests Inc.)

	Test	Source of test taxa
	Brachionus calyciflorus reproduction inhibition test (48hr EC_{50})	Rotoxkit F™ short-chronic kit (MicroBioTests Inc.)
Tests under refinement at UCEWQ	Indigenous algal (*Scenedesmus bicaudatus*, *Chlorella* sp.) growth inhibition test (96hr EC_{50})	Laboratory culture
	Caridina nilotica juvenile lethality test (96hr LC_{50})	Laboratory culture
	Caridina nilotica 10 day lethality test (10 day LC_{50})	Laboratory culture

All toxicity tests undertaken are listed in Table 3.1. The selected tests used taxa which were cultured under laboratory conditions, obtained from commercial toxicological kits, and collected from natural populations in the field. The taxa selected included both indigenous and non-indigenous taxa, with most test organisms being either macro-invertebrates or algal taxa. Toxicity tests included both acute (lethal) and chronic (sub-lethal) tests. All tests using commercial test kits followed the protocols devised for the kits. For most other toxicity tests, standard toxicity test protocols were followed (DWAF, 2000; Slabbert, 2004). Algal growth inhibition tests using axenic unialgal cultures established from algae collected from South Africa were all based on the *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) growth inhibition test (microplate assay) (Gola, in prep.). The tests using *Caridina nilotica* as a test organism are being developed at UCEWQ.

3.3 Results

Results of physico-chemical analyses of effluent samples (Table 3.2) show that the effluents assessed varied widely in both their physical and chemical properties. Table 3.2 also contains an indication, where applicable, of where chemicals exceeded either the General or Special Discharge Limits for discharge of waste or water containing waste into a water resource (DWAF, 2004). Colour interference indicates that the effluent was coloured (blue to black/brown) and this can potentially lead to interference with the toxicity tests, either in terms of being able to see the test organisms (especially smaller taxa) to ascertain endpoints (mortality or reproduction) or through interference with test results where optical density of samples (tests using algae and *T. thermophila*) or levels of luminescence (*V. fischeri*) were measured in order to assess sample toxicity. Neither filtration (GFC filter) nor centrifugation had any effect on coloured samples.

Table 3.2 Results of the physico-chemical analyses of samples of effluent from a wastewater treatment works (WWTW), a tannery, a dairy and a textile plant. * indicates that the sample exceeded both the General and Special Discharge Limits for discharge of waste or water containing waste into a water resource; ** indicates that the sample exceeded the Special Discharge Limits only (DWAF, 2004). BD indicates that results were below the test detection limits.

Water quality parameter	WWTW effluent	Tannery effluent	Dairy effluent	Textile effluent
pH	8.4**	3.3*	6.6	9.3**
Electrical conductivity ($mS \cdot m^{-1}$)	125.4**	176.69*	90.86	176.60*
Dissolved oxygen ($mg \cdot \ell^{-1}$)	8.2	1.42	0.71	1.21
Temperature (°C)	20.6	21.3	22.0	22.2
Colour interference	no	yes	no	yes
Nitrate ($mg\ N \cdot \ell^{-1}$)	9.6**	81*	6.3**	16.1*
Nitrite ($mg\ N \cdot \ell^{-1}$)	0.5	BD	BD	0.4
Ammonium ($mg\ N \cdot \ell^{-1}$)	4.4**	12.3*	0.2	0.2
Ortho-phosphate ($mg\ P \cdot \ell^{-1}$)	5.9**	34.6*	4.6**	3.6**
COD ($mg \cdot \ell^{-1}$)	BD	>10000*	3352*	1272*
BOD_5 ($mg \cdot \ell^{-1}$)	BD	50	2674	352

A summary of toxicity test results are presented in Table 3.3. Where endpoints as LC_{50} or EC_{50} could be derived, these are presented. Many tests did not produce valid LC_{50} or EC_{50} values. This was largely due to either excessive mortality or inhibition at the lowest effluent concentrations tested, or insufficient mortality or inhibition at the highest effluent concentrations tested (generally 100% except in some algal growth inhibition tests that used diluted effluent to avoid colour interference in optical density readings), or, in some cases, growth stimulation relative to controls.

In tests where excessive mortality or inhibition at low effluent concentrations was found, test repetition using more dilutions or a lower range of test effluent concentrations may have yielded valid endpoints. In many cases, tests were repeated, but the number of test repetitions was limited by the quantity of stored effluent available. As an extreme example, repeating the mayfly 10 day lethality tests would have required an extra 120 ℓ of stored effluent, and as a result these tests could not be repeated to assess the effect of tannery effluent at a concentration of less than 6.25%. Tannery effluent was most commonly associated with excessive mortality or inhibition, and, despite the lower ranges of tannery effluent concentration being *ca*. 0.01-0.4% in most tests, valid endpoints could not be derived. For most tests assessed here, tannery effluent can therefore be said to have an LC_{50} or EC_{50} of less than 0.4%.

All of the algal growth inhibition tests, with one exception, showed increased growth in effluents from a municipal wastewater treatment works and a dairy. This is most likely a response to elevated nutrient levels in these effluents. Although nutrient levels in the remaining effluents are generally as high or higher, algal growth was generally inhibited by these effluents. This is probably a function of other compounds in the remaining effluents.

Tests on textile effluent returned more valid endpoints than tests on any other effluent. This is at least partially due to the fact that the textile effluent is less toxic than the tannery effluent, but, unlike effluent from the wastewater treatment works and the dairy, did not result in growth stimulation in any of the tests.

Table 3.3 Summary of toxicological test results for all tests on effluents from a wastewater treatment works (WWTW), a tannery, a dairy and a textile plant. Data presented are EC_{50} or LC_{50} values as percent effluent. Where endpoints could not be derived, codes give an indication of test outcome*.

Toxicity test	WWTW effluent	Tannery effluent	Dairy effluent	Textile effluent
Daphnia pulex lethality test (laboratory culture) LC_{50}	ne	-e (<1.6)	8.8	nc
Daphnia pulex lethality test (Daphtoxkit F pulex kit) LC_{50}	nm	-e (<1.6)	nc	40.6
Daphnia magna lethality test (Daphtoxkit F magna) LC_{50}	-	-e (<0.8)	9.9	-
Daphnia pulex reproduction test (laboratory culture) EC_{50}	nc	nc	nc	nc
Caridina nilotica juvenile lethality test (laboratory culture) LC_{50}	1.5	-e (<6.3)	6.9	29.6
Caridina nilotica 10 day lethality test (laboratory culture) LC_{50}	-	nc	-	-
Mayfly 10 day lethality test (field collected Tricorythidae) LC_{50}	nm	-e (<6.3)	-	15.8
Mayfly 10 day lethality test (field collected Leptophlebiidae) LC_{50}	nm	-e (<6.3)		-
Pseudokirchneriella subcapitata growth inhibition test (laboratory culture) EC_{50}	+	-	+	-ve
Pseudokirchneriella subcapitata growth inhibition test (Algaltoxkit F kit) EC_{50}	+	-e (<0.01)	+	6.4
Scenedesmus bicaudatus growth inhibition test (laboratory culture) EC_{50}	ne	-	+	ne
Chlorella sp. growth inhibition test (laboratory culture) EC_{50}	+	nm	+	-ve
Vibrio fischeri bioluminescence test (Biotox kit) EC_{50}	+	0.3	-	48.6

Toxicity test	WWTW effluent	Tannery effluent	Dairy effluent	Textile effluent
Tetrahymena thermophila growth inhibition test (Protoxkit F kit) EC_{50}	-ve	ci	-ve	-e (<6.3)
Brachionus calyciflorus reproduction inhibition test (Rotoxkit F short-chronic kit) EC_{50}	4.3	-e (<6.3)	-e (<6.3)	-e (<6.3)

* -: limited mortality/inhibition at highest effluent concentration.
-e: mortality/inhibition at lowest effluent concentration too high for endpoint derivation. Lowest effluent concentration assessed in parentheses.
+: effect stimulation at highest effluent concentration.
-ve: inhibition/mortality at low effluent concentrations, with impact decreasing in higher effluent concentrations.
nc: control mortality, growth or reproduction exceeds limits for test validity.
nm: non-monotonic dose response curve precludes endpoint derivation.
ne: effluent has no effect at any concentration tested.
ci: colour interference by effluent precludes endpoint derivation.

It was not possible given the results presented in Table 3.3 to directly compare the sensitivities of the *D. pulex* lethality tests using the commercial Daphtoxkit F pulex kit and the test using laboratory cultures for test organisms as no effluent produced comparable LC_{50} endpoints. No clear toxicological response was detected for either test using wastewater treatment works effluent. Both tests showed excessive mortality when exposed to even 1.6% tannery effluent. For the remaining two effluents, one test in each case did not return a valid result owing to high control mortalities. However, from the mortality data returned, the LC_{50} of the test using the commercial kit on dairy effluent seems likely to fall in the range 6.3-12.5%, which is comparable to the results of the test using laboratory cultures as a source of the test organism. In the same way, the LC_{50} of the test using laboratory cultures on textile effluent seems likely to fall in the range 25-50%, comparable to the results of the test using the commercial kit.

The response of the *D. magna* lethality test to the effluents tested is similar to that of the *D. pulex* lethality test for the endpoints returned.

When the results of the *P. subcapitata* growth inhibition test using the commercial kit and the results of the same test using test organisms from laboratory culture were compared, both were found to show growth stimulation on exposure to effluent from the municipal wastewater treatment works and the dairy. For the remaining two effluents, it is important to note that the range of effluent concentrations tested varied between the tests using the commercial kits and the tests using laboratory cultures. In the case of tests using commercial kits, effluent concentrations over the ranges 0.01-100% (tannery effluent) and 1.6-100% (textile effluent) were tested. In both cases, though especially in tannery effluent, colour interference complicated assessment of algal yield, particularly at higher effluent concentrations. As a result of this, the tests using laboratory cultures of *P. subcapitata* (and other indigenous algae) used an effluent concentration range of 0.4-6.3% for assessment of toxicity owing to tannery and textile effluents. In the test of tannery effluent using a commercial kit, inhibition decreased as the concentration of effluent decreased below 1.0%, and the results suggest an EC_{50} of less than 1.0% and possibly less than 0.01%. In the test using laboratory cultures of *P. subcapitata*, the results suggest an EC_{50} in the range

0.4-0.8%. In neither of these examples are the conditions for valid endpoint derivation met, however. In the case of textile effluent, the test using a commercial kit returned an EC_{50} of 6.4%, but, due to the loss of replicate treatments, this cannot be considered as definitive. In contrast, little can be drawn from the results of the test using laboratory cultures as a source of test organisms, as the inhibition found at 0.4% effluent decreased steadily with increasing effluent concentration (until 6.3%, the highest concentration assessed).

Responses of the indigenous algal taxa to the four effluents varied. With the exception of *S. bicaudatus*, all showed growth stimulation in response to wastewater treatment works and dairy effluent. *Scenedesmus bicaudatus* also showed no response to textile effluent, while *Chlorella* sp., like *P. subcapitata*, was inhibited by this effluent, though with no clear trend across the concentration range assessed. Both taxa were inhibited by tannery effluent across the range of concentrations assessed, though again with no clear trend in response to concentration. Both indigenous taxa seemed in general less sensitive to the effluents assessed than *P. subcapitata*.

The *Caridina nilotica* juvenile lethality test, using an indigenous freshwater shrimp as a test organism, proved the most tractable of the tests assessed in this study, as it returned LC_{50} endpoints for three of the four effluents assessed. Owing to constraints on test repetition due to test effluent availability, this test was not repeated using a lower range of tannery effluent concentrations, and so, for this remaining effluent, one can only state that results suggest an LC_{50} value of less than 6.3%. When compared to other tests that returned valid endpoints, the *C. nilotica* juvenile lethality test is revealed to be the most sensitive test when assessing the toxicity of wastewater treatment works effluent and dairy effluent, and the third most sensitive when assessing the toxicity of textile effluent. Those tests that did not return endpoints as EC_{50} or LC_{50} because responses were insufficient at the highest concentrations of toxicants assessed can generally be considered as less sensitive than the *C. nilotica* juvenile lethality test.

In contrast to the *C. nilotica* juvenile lethality test, the *C. nilotica* 10 day lethality test returned no valid endpoints. After initial testing indicated excessive mortality over the concentration range assessed, all tests were undertaken using a lower range of concentrations. While mortality in response to all effluents was noted in the latter tests, responses to effluents were for various reasons inappropriate for endpoint derivation.

The *V. fischeri* bioluminescence test returned two valid endpoints for the four effluents assessed, making this the second most tractable of the tests assessed here. When the endpoints from this test are compared with other test results, they suggest that this test is perhaps the least sensitive of the tests assessed with respect to the effluents under study.

Beyond the *C. nilotica* juvenile lethality test and the *V. fischeri* bioluminescence test, no test produced more than one valid and precise endpoint for the four effluents assessed. When excessive mortality even at low concentrations of effluent was observed, one can state that the endpoint, as LC_{50} or EC_{50}, lies below the lowest concentration tested, provided that other conditions for test validity are met. Where growth stimulation was observed across a range of concentrations increasing to 100%, no toxicological endpoints could be determined. Where inhibition was recorded, it is likely that further repetition of tests may in some cases have yielded valid LC_{50} or EC_{50} endpoints. Alternatively, selection of another endpoint may have provided a better indication of effluent toxicity.

Several of the tests assessed here proved difficult to apply. No results were returned from the *D. pulex* reproduction test as a result of problems in producing viable neonates in sufficient number to be used for the test. An acute toxicological response to tannery, dairy and textile effluent precluded assessment of reproductive inhibition using the *B. calyciflorus* reproduction inhibition test. The *T. thermophila* growth inhibition test showed an inverse response to effluent concentration when testing wastewater treatment works and dairy effluent, while a result for tannery effluent was precluded by colour interference with optical density readings. As noted above, the mayfly 10 day lethality test required 120 ℓ of effluent which precluded any test repetition should the initial dilution series selected prove inadequate for endpoint derivation. Nevertheless, this test, when using Tricorythid mayflies, was one of the more sensitive when assessing the toxicity of textile effluent.

In summary, despite the problems encountered, the toxicity test results showed that there are differences in responses of the various test taxa to exposure to the effluents. Tannery effluent was overall the most toxic of the effluents assessed, but test organism responses vary across the remaining effluents. In as far as could be determined, the results from the *D. pulex* lethality tests undertaken using a commercial kit and test organisms from laboratory culture are comparable. Owing to differing implementations, it is less clear how the results from the *P. subcapitata* growth inhibition tests using a kit and test organisms from laboratory culture compare.

3.4 Discussion

Currently, standard methods for undertaking aquatic whole-organism toxicity tests depend heavily on the use of taxa from laboratory cultures. However, given the pressures on laboratory resources and the costs of culture maintenance (Persoone and Van de Vel, 1988), it is not always feasible to maintain such cultures. With this in mind, methods have been developed that utilize the ability of various organisms to produce dormant or resistant stages in their life history, and these stages, once reinvigorated, provide tests organisms for the assay. This approach underlies the majority of commercial whole-organism toxicological test kits. This has, however, led to various questions regarding the sensitivity of such test organisms, and their performance in test sensitivity and repeatability when compared with standard culture-based tests. When the endpoints produced by standard tests using cultured test organisms have been compared with endpoints from commercial kits, the consensus seems to be that the commercial kits make good surrogates for standard tests (Blaise, 2000; Janssen et al., 2000; Daniel et al., 2004; Persoone et al., 2009; Persoone and Wadhia, 2009). However, not all authors completely concur (e.g. Jung and Bitton, 1997). Lucivjanská et al. (2000) noted differences in the responses of *D. magna* from kits and the standard culture-based test that were revealed to be a function of differing water hardness owing to an error in the amount of calcium chloride in the growth medium distributed with the kit, and were not due to fundamental differences between the kit and standard culture-based tests. Most published comparisons of commercial kits and standard tests have focussed on the algal (*P. subcapitata*) growth inhibition test and the *D. magna* lethality test, both of which formed part of this project, and most authors found sufficient concurrence between endpoints from kits and standard methods. This study however included comparisons of kits and standard methods using *P. subcapitata* and *D. pulex*, rather than *D. magna*, as test organisms. Direct comparison between kits and standard tests was complicated by difficulties in deriving endpoints for all assays undertaken. Consequently, a comparison of the results for the same test using kits and standard culture-based tests was inconclusive.

Further research in which endpoints are obtained for all tests using a suite of test effluents is necessary to more accurately quantify the relation between test methods using kits and standard culture-based methods. Problems encountered in obtaining endpoints in a range of effluents are further considered below.

The results obtained here indicate differences in sensitivity between the various test organisms. This is in common with findings from other studies. For example, Daniel et al. (2004) compared the results of toxicity tests of 38 effluent samples using two commonly used regulatory species and seven commercially available toxicity test kits. Although the taxa used were not the same as those tested here, the final assessment indicated that each of the assays demonstrated both advantages and disadvantages. These results are similar to those found in the current study. Fochtman et al. (2000) assessed the effect of 13 pesticides on 7 test taxa, and found on analysis that 82% of the variation in responses could be attributed to two main effects: tests using crustacean and fish taxa responded similarly, but unlike tests using algal and protozoan taxa, which were similar to each other. The authors note a differentiation in the responses of single-celled and larger test taxa, and used their results to recommend a suite of four tests for use in testing pesticide toxicity. In a similar way, Pandard et al. (2006) examined the responses of six assays when used on 40 wastes. They too found that a smaller battery of tests could represent the six tested, but found that the tests were separated largely based on the type of toxicological test rather than the test organism. All these studies indicate the need for the use of a suite of carefully selected tests with a range of test taxa to best characterize toxicants. Chemical analysis alone does not capture all the information returned by toxicological testing, though is useful in conjunction with toxicological testing (Latif et al., 1995; Farré and Barceló, 2003; Manusadžianas et al., 2003; Mendonça et al., 2009; Wolska et al., 2007). It would therefore appear prudent for laboratories to select several from the most appropriate technologies and methods for their application, as each of the tests used has advantages and disadvantages.

Test results presented here revealed interesting differences in sensitivity between indigenous and standard test taxa. Although the decision has been made to focus on a suite of well established toxicity tests which have many years of international research backing them (DWAF, 2003; DWAF, 2005), the question of the relevance of including indigenous taxa for site specific water quality management remains. It may be necessary, in future, to reassess the suite of species that are included in toxicity testing for regulatory purposes. Of the native taxa assessed here that are not standard test organisms, *C. nilotica* (in the *C. nilotica* juvenile lethality test) seems promising as more valid endpoints were obtained compared to other tests assessed, and most of these were obtained using the initial dilution series. In addition, the test proved fairly sensitive to a range of effluents. In contrast, however, the *C. nilotica* 10 day lethality test returned no valid endpoints. This was however a function of not using an appropriate dilution series for testing, even with test repetition. Tests with mayflies were not easy to practically implement or repeat owing to the large quantity of effluent required for each test. As well as logistical challenges in sampling and storing such quantities of effluent, subsequent disposal of the effluent may be problematical, particularly if the effluent is highly toxic. The South African algal isolates did not seem more sensitive than the standard test organism *P. subcapitata* to the effluents assessed. With one exception, all algal tests showed growth stimulation in response to two of the effluents tests. The remaining effluents were problematical to work with owing to their colour cast.

From the results, it can be quite clearly seen that not all tests were successfully carried out, and that effect values (either lethal concentrations or effect concentrations) could not always be computed. This is not unique: Daniel et al. (2004) report on a large-scale testing of 38 effluents using 9 assays, and were unable to produce EC_{50} values for many of the tests. The toxicity tests undertaken in the current study were carried out in a research laboratory by experienced researchers, and yet many problems were encountered requiring repeats of toxicity tests. Issues were varied, ranging from the concentration ranges selected, which were based on the recommendation in the DEEEP manual (Slabbert, 2004), being either too high (effluents were highly toxic) or "too low" (effluents were not acutely toxic) to managing the mortalities of organisms in the controls, particularly during longer exposure periods. During tests where control mortalities are high, tests should be repeated. However, repetition of toxicity tests until a definitive result was obtained was not practically feasible for this project, and laboratories undertaking regulatory toxicity testing may find themselves in similar positions. As regulatory toxicity testing becomes mandatory, and laboratories are established, this needs to be considered, particularly where unknown effluents are encountered. A further consideration is the training level of staff to undertake the toxicity tests: at times, critical decisions regarding the toxicity tests will need to be taken and it is vital that staff undertaking the test are sufficiently skilled to make key decisions, such as what to do next when the effluent does not present any acute toxicity even at the highest effluent concentration. As with the results reported by Daniel et al. (2004), some of the effluents tested for this project were not acutely toxic, or as toxic as expected. As this is a common situation, there should be clearly developed guidelines as to what to do next.

The effluent itself may also present challenges for the interpretation of organism responses during the test, particularly if it is coloured. Not only can it be difficult to see smaller organisms, but there may be interference with reading of luminescence or optical density in those assays that require this. Although at higher dilutions it becomes easier to see the organisms and there is less optical interference, it does beg the question of the effects of chemical speciation on the toxicity test results: as the dilution range changes, the chemical composition of the effluent is also affected, and this may result in changing chemical speciation. Some of the challenges encountered in obtaining results for this project may possibly be attributed to chemical speciation as it was difficult for some tests to find a suitable concentration range that would yield an effect value. This was particularly evident where results from acute toxicity tests were used to predict the concentration range for chronic toxicity tests (e.g. use of the *D. pulex* lethality test result to determine the concentration range for the *D. pulex* reproduction test). It may be more appropriate when testing coloured whole effluent to use a test that does not require dilution of the sample, such as one of those using *Lemna* spp as a test organism (Römbke et al., 2009).

There are many research questions that have arisen from this short project and some are listed here. This list is by no means exhaustive, but provides an insight into some of the key questions which need to be addressed in order to effectively implement and utilize these tools of toxicity testing in water resource management.

Experience from this research project has shown that there are potentially many sources of error in undertaking regulatory toxicity testing. It is vital that further refinement of the test methods, with explicit statements on how to handle specific circumstances and situations of effluent quality need to be incorporated, especially as there is currently not a large body of experienced toxicologists in South Africa. Methods need to be explicit, allowing no room for

interpretation of methods. And experience needs to be developed, so that toxicologists undertaking the tests gain insight as to what to do when “things just go wrong”.

It is imperative that the necessary resources needed to undertake routine and regulatory toxicity testing be developed in South Africa. These resources are, specifically, human resources, together with laboratory facilities where technicians can become trained and skilled in the scientific methods required for undertaking toxicity tests. Regulatory toxicity testing is set to become a key tool in managing environmental water quality, and so the necessary resources to perform these tests need to be developed as a matter of urgency. Increasing the number of facilities to undertake the toxicity tests will lead to increased data availability and subsequent research questions and refinement and development of more appropriate toxicity test methods can be undertaken.

Although there currently is a trend in the international literature for the application of toxicity testing using commercial toxicity test kits (Blaise, 1998; Blaise, 2000; Dewhurst et al., 2002; Mitchell et al., 2002; Manusadžianas et al., 2003; Daniel et al., 2004; Wadhia and Clive Thompson, 2007; Wadhia and Persoone, 2009), South Africa is still facing the challenges of implementing toxicity tests per se, across different trophic levels. There is an urgent need to implement toxicity testing through DEEEP and the NTMP, in order to better understand how these toxicity tests can be optimally used in managing water quality in surface water resources. It is recommended that further testing using a wide range of effluents be undertaken, either using assays based on laboratory cultures or commercial toxicity test kits. This further testing will allow for further refinement of methods (if necessary) and/or improvements to current methods manuals. Interpretation of results of these toxicity tests and subsequent understanding of the impacts of effluent on environmental water quality are key for successful implementation of regulatory toxicity testing. Research questions such as the ecological impacts of the effects of stimulation rather than inhibition in the algae and bacterial toxicity tests need to be addressed, as well as examining whether laboratory toxicity tests are adequate to predict large-scale ecological consequences. There is also an urgent need to investigate the use of biochemical biomarkers as early warning indicators of potential toxic effects.

4 RELATIVE COSTS OF TOXICOLOGICAL TESTING USING COMMERCIAL TEST KITS AND STANDARD TESTS WITH CULTURED AND WILD-COLLECTED ORGANISMS

4.1 Introduction

Given that a particular toxicological test used to evaluate whole effluent toxicity has both ecological relevance and generates reproducible and defensible endpoints, the choice of test, or tests, will be influenced by test costs. Test costs are determined by the costs of equipment and consumables for testing as well as time and skill requirements to undertake the tests. Where cultures are maintained for the production of test organisms, the costs of culture maintenance add to the overall cost per test.

Tests used for toxicity testing have changed over time and a trend can be identified from larger tests that often used cultured test organisms through to more cost-efficient, rapid, microscale tests (Blaise, 1998). Many of the latter tests are available as commercial kits. Manufacturers of commercial toxicological test kits claim that their products are “culture/maintenance free, simple, rapid, robust, sensitive, reproducible, [and] low-cost “ (Microbiotest Inc, 2009). Many of these claims are examined elsewhere in this report. This chapter will assess whether the kits are relatively cheap, by examining the cost implications of equipment and consumable requirements of tests using kits and standard toxicity tests, along with labour requirements for testing. The cost implications of maintaining cultures of test organisms will also be examined.

Toxicological test kits are designed to be easily and rapidly deployable. One of the ways that this is achieved is to remove reliance on sufficiently large and healthy cultures of test organisms. Maintenance of cultures has been identified as a significant overhead on standard toxicological testing, particularly when fewer tests are undertaken (Persoone and Van de Vel, 1988). Avoiding relying on cultures for testing is particularly useful when testing is rare, infrequent or at unpredictable intervals, all cases where continued upkeep of cultures of sufficient size would constitute a considerable burden.

Standard tests using indigenous organisms may have greater ecological relevance than tests using standard organisms. However, they may have greater requirements in terms of skills, time, equipment and consumables than tests using kits, particularly when cultures of test organisms need to be maintained. Where kits using indigenous organisms are available, this is not an issue. However, a number of potential test organisms are not suited to use in kits owing, for example, to the lack of a dormant stage in their life history.

This cost analysis will examine the cost implications of toxicological testing using commercial test kits from two suppliers and will compare the costs incurred with the costs of toxicological testing using cultured and wild-collected test organisms. The analyses will examine several scenarios in an attempt to identify areas where costs may be rationalized when equipping a laboratory for toxicity testing. The results of the cost analyses will be compared with quotes from external laboratories so as to identify circumstances where outsourcing of testing might be more cost-efficient, or when it might be more cost-efficient to undertake tests in-house using either commercial kits or cultured or wild-collected test organisms.

4.2 Methodology

Tests assessed

The tests that were assessed to determine the cost implications of implementing them are given below. The commercial toxicological test kits assessed are presented in Table 4.1. All kits were obtained from Toxsolutions Kits and Services[1].

Table 4.1 Commercial toxicity test kits assessed for cost efficiency along with the manufacturer of the kit and the test organism used.

Kit	Manufacturer	Test organism
Algaltoxkit F™	MicroBioTests Inc.	*Pseudokirchneriella subcapitata*
Biotox	Aboatox Oy	*Vibrio fischeri*
Daphtoxkit F™ magna	MicroBioTests Inc.	*Daphnia magna*
Daphtoxkit F™ pulex	MicroBioTests Inc.	*Daphnia pulex*
Protoxkit F™	MicroBioTests Inc.	*Tetrahymena thermophila*
Rotoxkit F™ short-chronic	MicroBioTests Inc.	*Brachionus calyciflorus*

Toxicological tests using cultured or wild-collected organisms that were assessed for cost implications are given in Table 4.2. In most tests assessed in this report, cultures were maintained for the supply of test organisms. The mayfly 10 day lethality test is unusual amongst the tests assessed here in that test organisms are collected in the wild, rather than cultured or sourced along with test kits. The assumption is made that accessible wild populations are sufficient to sustainably support collection of these organisms for tests.

Table 4.2 Tests using either cultured or wild-collected organisms that were assessed, and the sources of the test organisms.

Test	Source of test organism
Algal (*Pseudokirchneriella subcapitata*) growth inhibition test	*Pseudokirchneriella subcapitata* culture
Algal (*Scenedesmus bicaudatus*) growth inhibition test	*Scenedesmus bicaudatus* culture
Algal (*Chlorella* sp.) growth inhibition test	*Chlorella* sp. culture
Caridina nilotica juvenile lethality test	*Caridina nilotica* culture
Caridina nilotica 10 day lethality test	*Caridina nilotica* culture
Daphnia pulex lethality test	*Daphnia pulex* culture
Daphnia pulex reproduction test	*Daphnia pulex* culture
Mayfly 10 day lethality test	Wild collection of Ephemeroptera

1 ToxSolutions, PO Box 682, Irene 0062, South Africa

Costs assessed

The requirements per test in terms of equipment items, consumables and labour for all toxicological tests assessed were determined. The protocols that are followed at the Unilever Centre for Environmental Water Quality (UCEWQ) were used to draw up lists of requirements for tests using cultured or wild-collected organisms, and the protocols followed by Toxsolutions Kits and Services were used to tabulate requirements for the tests using commercial toxicological kits. Costing of culture maintenance was based on the culture facilities maintained at UCEWQ.

Itemized lists of all test requirements are presented in Appendix A. All costs were classified as follows:

Equipment: capital and other equipment that is re-used from test to test or for ongoing culture maintenance.

Consumables: items that are required for tests or cultures that are not re-used. This category includes reagents that are used during the test as well as disposable equipment.

Labour: this is calculated from estimates of the amount of time required to complete one test, or to maintain a culture for one month. Two pay grades are considered in this analysis, and the various tasks needed to complete a test are assigned to one or other of the pay grades, depending on skill requirements. Only time spent engaged in test or culture-specific tasks is considered. More general support tasks are less easily defined and have therefore been excluded from this analysis.

The costs of equipment and consumables are based on quotes from suppliers obtained during 2008. All costs are VAT exclusive. Costs for electricity, tap water and laboratory rental or purchase were not included in this analysis.

Labour costs are based on an analysis of time and skill requirements for the various tests or cultures. Two skill levels were used in this analysis, with the majority of the work associated with testing or culture maintenance being assigned to a technical assistant, while data analysis and supervision were undertaken by a technical officer. Remuneration rates for these pay grades are derived from Rhodes University pay scales for these ranks at the start of 2009. Remuneration rates were taken as the middle of the ranges for pay grade 6 (technical assistant) and pay grade 7 (technical officer).

Costs of undertaking tests were compared with the costs of outsourcing testing to external laboratories. The data on costs of outsourcing tests are based on quotes from commercial laboratories obtained between November 2007 and November 2008. One quote was corrected after this period and the updated quotes were adjusted for inflation since June 2008, assuming inflation at 10% per year. All quotes are VAT exclusive.

Costing approach

Several approaches are taken to assess the costs associated with each test or groups of tests. Firstly, basic costs of tests and cultures are calculated. Secondly, the potential for savings where more than one procedure may share equipment is considered. Finally, the implications for the cost per test of any one test owing to re-use of equipment is assessed.

Costs due to equipment depreciation and replacement and repair of equipment are not considered as part of this analysis. This is largely as the rate of depreciation and breakage is not easily defined meaningfully and simultaneously in the context of cultures, where costs are calculated on a monthly basis, and in the context of tests, where costs are calculated on a per-test rate. For the sake of simplicity these costs are omitted.

Outsourcing of testing

Several independent external laboratories were approached for quotes to undertake the tests recommended under DEEEP in order that costs of testing complex effluents using commercial toxicological test kits and standard tests using cultured or wild-collected organisms could be compared with the costs that would be incurred by outsourcing of testing.

Overall costs per test/per culture

The cost of the equipment, consumables and labour required for each test were calculated. The costs of maintaining a culture were calculated on a monthly basis, and, based on the maximal rate of testing for each culture, the contribution of culturing test organisms to the overall test cost was derived. The maximum capacity of culture facilities was taken to be that of the culture facilities and laboratories of UCEWQ as they stood at time of analysis. These are given in Table 4.3.

Table 4.3 Maximum rate of testing for tests that use cultures at UCEWQ laboratories.

Culture	Test	Maximum test rate (month^{-1})
Pseudokirchneriella subcapitata	Algal (*Pseudokirchneriella subcapitata*) growth inhibition test	20
Scenedesmus bicaudatus	Algal (*Scenedesmus bicaudatus*) growth inhibition test	20
Chlorella sp.	Algal (*Chlorella* sp.) growth inhibition test	20
Caridina nilotica	*Caridina nilotica* juvenile lethality test	4
	Caridina nilotica 10 day lethality test	4
Daphnia pulex	*Daphnia pulex* lethality test	4
	Daphnia pulex reproduction test	4

Potential savings due to shared equipment

Several items of capital equipment are used in more than one test. In some cases, these equipment items are a major part of the cost of introducing a new test in a laboratory. In addition, a large quantity of standard laboratory equipment can be shared between tests. We analyzed the costs of introducing new tests to a laboratory undertaking either tests using commercial kits or tests using cultured or wild-collected organisms in order to identify tests that had unique equipment requirements, as well as tests where sharing of equipment might

reduce the capital cost of introducing a new test. Where items of equipment were dedicated to a test or to a culture and could not be shared, they always contribute to the cost of the test or culture.

In the case of tests using commercial kits, unique equipment requirements of any one test were evaluated in comparison to the equipment requirements of the other five kits assessed.

Tests using cultured organisms require that a culture of the test organism be maintained. The cost of maintaining such cultures adds to the cost of the test itself. The following three approaches were taken to identifying the unique costs of tests using cultured and wild-collected organisms:

- The cost of adding equipment for any one test to a laboratory where equipment for the other tests assessed in this analysis was present. For the purpose of this analysis, the two tests using algae are treated as a single group as equipment requirements for testing are identical. Savings due to sharing of equipment in the test being assessed with equipment used for culturing of any of the test organisms is not considered.

- The cost of adding any one of the cultures assessed here to a laboratory where equipment for culturing all of the remaining cultured test organisms is present. For the purposes of this analysis, the two algal cultures are treated as a single group as equipment requirements for culturing are identical. Savings due to sharing of equipment between the culture being assessed and any of the test procedures is not considered in this analysis.

- The cost of adding all tests using a cultured or wild-collected test organism, and a culture of that test organism, is compared with other culture/test groups. This comparison was initiated as many of the costs associated with a new test or culture are shared with other tests using that organism. This means that should a laboratory choose to start culturing a test organism for one test, a second test using the same organism could be offered at very little extra cost. For the purposes of this analysis, the two algal cultures are treated as a single group (algal tests) as equipment requirements for culturing and testing are identical. Otherwise, groups assessed are tests using *D. pulex* and *D. pulex* cultures, tests using *C. nilotica* and *C. nilotica* cultures, and tests using mayflies.

Decreasing cost per test as tests are repeated

A large part of the cost of many of the tests assessed in this report is the cost of large items of capital equipment required for the test (or associated culture). This analysis looks at the rate that cost per test decreases as the test is repeated and capital equipment is re-used.

As this analysis uses information on costs of tests and, where appropriate, also includes costs of cultures, it was necessary to include an assumption about the rate of testing so that fixed costs per sample and costs of maintaining cultures per month could be meaningfully combined. For the purposes of the analysis, it was assumed that tests were run at the maximum capacity of the culture facilities (Table 4.3).

4.3 Results

Costs of outsourcing tests

For the purposes of comparison, it is important to note that the alga used in the algal (*Selenastrum capricornutum*) growth inhibition test mandated under DEEEP is correctly known as *Pseudokirchneriella subcapitata*. The algal (*S. capricornutum*) growth inhibition test mandated under DEEEP is the same test as the algal growth (*P. subcapitata*) inhibition test assessed here as one of the tests using cultured test organisms. The test using the Algaltoxkit F assessed here as one of the commercial kits is a very similar test that also assesses changes in the growth of *P. subcapitata* on exposure to a potential toxicant over 72 hours.

The ranges of costs for tests quoted by commercial laboratories are presented in Table 4.4. No single commercial laboratory was able to undertake all the tests mandated under DEEEP. Of the five laboratories approached, none indicated any capacity to routinely undertake the Ames *Salmonella* mutagenicity test, though one laboratory suggested replacing the Ames test with another test that used a bacterium as the test organism, the *Vibrio fischeri* acute test. As the Ames *Salmonella* test was included in DEEEP to provide a measure of mutagenicity and not simply short-term toxicity (DWAF, 2003), the *V. fischeri* test is not an adequate substitute.

Table 4.4 Range of prices for toxicological tests as quoted by five independent external laboratories. No laboratories offered a quote for the Ames *Salmonella* test, and one suggested the *Vibrio fischeri* acute test as a substitute. All costs are VAT exclusive.

Test	Quoted costs
Chemical oxygen demand (COD)	R64-103
Biochemical oxygen demand (BOD)	R211-231
Fish (*Poecilia reticulata*) lethality test	R1 155-1 248
Daphnia pulex lethality test	R921-1 366
Daphnia pulex reproduction test	R5 765-6 976
Ames *Salmonella* mutagenicity test-plate incorporation assay	N/A
Algal (*Selenastrum capricornutum*) growth inhibition test	R1 566-1 914
Vibrio fischeri acute test	R842

The more routine COD and BOD tests cost significantly less than any of the toxicological tests. The cheapest of the toxicological tests was the *V. fischeri* acute test, and costs per test increased gradually through the *D. pulex* lethality test to the fish (*Poecilia reticulata*) lethality test and then to the algal (*S. capricornutum*) growth inhibition test. The *D. pulex* reproduction test was significantly more expensive than the other toxicological tests.

Overall costs per test/per culture

The costs presented in this section deal mostly separately with the costs of testing and culture maintenance, though the combined costs of undertaking tests that use cultures and culture maintenance for those tests are considered later. All equipment, consumables and time requirements used to calculate the costs presented below are listed in Appendix A.

The costs of equipment required to undertake a test using cultured or wild-collected organisms, as well as the costs of labour and consumables needed to undertake one test are presented in Table 4.5. Of the tests assessed here, the algal growth inhibition tests, which have identical equipment requirements, have the largest equipment requirements. Both tests using *D. pulex* as a test organism have relatively high equipment requirements, and, though the equipment required is less than that required for the algal growth inhibition tests, it is greater by an order of magnitude than the equipment requirements of the *C. nilotica* juvenile lethality test.

Table 4.5 Overall cost of the toxicological tests using cultured and wild-collected organisms that are assessed in this report. Equipment costs reflect the costs of equipment required to undertake a test. Consumable and labour costs are expressed on a per test basis. The total cost per test is also presented. All cost estimates are based on the protocols of UCEWQ laboratories. All costs are VAT exclusive.

	Start-up cost	**Cost per test**		
	Equipment	**Consumables**	**Labour**	**Total**
Daphnia pulex lethality test	R224 138	R72	R380	R452
Daphnia pulex reproduction test	R224 738	R87	R1 810	R1 897
Mayfly 10 day lethality test	R34 503	R173	R1 971	R2 144
Algal (*Pseudokirchneriella subcapitata*) growth inhibition test	R378 604	R231	R228	R459
Algal (*Scenedesmus bicaudatum*) growth inhibition test	R378 604	R231	R228	R459
Algal (*Chlorella* sp.) growth inhibition test	R378 604	R231	R228	R459
Caridina nilotica juvenile lethality test	R23 565	R58	R392	R450
Caridina nilotica 10 day lethality test	R34 335	R88	R550	R638

All of the tests in Table 4.5 that have high equipment costs use ultrapure water during the test. A water purifier is therefore part of the equipment for these tests. This single item of equipment accounts for approximately half or more of the total equipment cost of these tests. Excluding a water purifier, the other equipment costs of tests using *D. pulex* are only approximately R43 000. As the equipment costs listed in Table 4.5 reflect the costs of setting a laboratory up from scratch, it is clear that considerable savings can be made should such

an expensive piece of equipment already be present, or if an alternate, cost-efficient source of ultrapure water was available.

The most expensive by far of the tests assessed are the algal (*P. subcapitata*, *S. bicaudatum* and *Chorella* sp.) growth inhibition tests. These tests require a number of specialized and costly pieces of equipment. However, the costs per test are relatively low. Once this procedure is established in a laboratory, there is also the potential for a high turnover of samples (Table 4.3) and the initially high start-up costs might be recouped. This is in distinct contrast to the mayfly 10 day lethality test, which has relatively low equipment requirements, but a high cost per test. Without the purchase of more equipment, sample turnover using the mayfly 10 day lethality test is relatively low as a maximum of four samples could be processed every month with current equipment.

The start-up equipment requirements of the tests using mayflies and *C. nilotica* are relatively low. This is largely because little specialized equipment is required. Much of the equipment required for these tests is standard laboratory fare, and the rest can largely be sourced from suppliers to aquarists, hardware stores *et cetera*.

A major difference between tests is the amount of time required to process one sample, and the consequent labour costs. Two tests, the *D. pulex* reproduction test and the mayfly 10 day lethality test, require considerably more time to run, and this contributes significantly to the cost of running a test. The two algal growth inhibition tests are at the other end of the spectrum. Here, assessment of samples required little labour and consumables, rather than labour, contribute significantly to the cost of testing one sample. In all tests bar the algal growth inhibition tests, labour costs make up the major part of the cost of processing a sample.

The start-up equipment costs and the costs of processing one sample with tests using commercial kits are presented in Table 4.6. Though start-up equipment costs are significant, none of the tests using commercial kits have greater equipment costs than the more expensive of the tests using cultured and wild-collected organisms. This is largely because the cost of a water purifier, required for some of the tests using cultured and wild-collected organisms, was high. When this cost is ignored, the equipment costs of tests using cultured and collected organisms, with the exception of the algal growth inhibition tests, range from R24 000 to R43 000. This is less, generally, that the equipment costs of tests using commercial kits.

Table 4.6 Overall cost of the toxicological tests using the commercial test kits that were assessed in this report. Equipment costs reflect the costs of equipment required to undertake a test. Consumable and labour costs are expressed on a per test basis. The total cost per test is also presented. All cost estimates are based on protocols used at the laboratories of Toxsolutions Kits and Services. All costs are VAT exclusive.

	Start-up cost	**Cost per test**		
	Equipment	**Consumables**	**Labour**	**Total**
Algaltoxkit F	R91 985	R1 129	R118	R1 247
Daphtoxkit F magna	R40 659	R479	R90	R568
Daphtoxkit F pulex	R40 659	R396	R90	R485
Rotoxkit F short-chronic	R53 717	R811	R185	R995
Protoxkit F	R99 335	R677	R110	R787
BioTox	R115 749	R390	R110	R500

The overall cost per test of tests using cultured and collected organisms ranges from R2 144 to R450. Three of these tests have overall costs per test of approximately R450. The next most expensive test is significantly more at R638, and the remaining two tests cost roughly R2 000 per sample. In contrast the costs involved in testing one sample using commercial kits range from R485 to R1 247. The smaller range of costs of the tests using commercial kits means that both the cheapest and most expensive of the tests, on a per-sample basis, were tests using cultured and collected organisms. The cheapest tests are the four tests using cultured and collected organisms that cost approximately R450 per test: the *D. pulex* lethality test, the two algal growth inhibition tests and the *C. nilotica* juvenile lethality test. The most expensive tests were the two most labour-intensive: the *D. pulex* reproduction test and the mayfly 10 day lethality test. Of tests using the commercial kits, the cheapest are tests using the Daphtoxkit F pulex kit, the Biotox kit, and the Daphtoxkit F magna kit. The most expensive of the tests using kits are tests using the Algaltoxkit F kit and the Rotoxkit F short-chronic kit.

In an attempt to compare similar tests, the costs of the *D. pulex* lethality test using cultured *D. pulex* and the test using the Daphtoxkit F pulex kit may be compared. The cost per test is similar for these tests. If an alternate source of ultrapure water was available for the test using cultured *D. pulex*, the costs of equipment are at least fairly similar. In the same way, the algal (*P. subcapitata*) growth inhibition test can be compared with the test using the Algaltoxkit F kit. Here, the cost per test of the commercial kit is far higher than the test that uses live cultures. However, the cost of start-up equipment for the test using cultured *P. subcapitata* is far higher than start-up equipment costs for a test with the commercial kit. Even if an alternate source of ultrapure water was available for the test using cultured *P. subcapitata*, the equipment requirements remain high and more expensive than those of the test using the Algaltoxkit F kit.

A general trend noted in the cost per test of the commercial kits is the relatively low labour costs. In contrast, consumable costs were relatively high. Nearly all the consumable costs of commercial kits are attributable to the cost of the kits themselves. This is in contrast to the

tests using cultured or collected organisms, where costs per sample were largely due to labour costs. The extreme is seen when comparing the mayfly 10 day lethality test and the Algaltoxkit F: the former is relatively expensive at R2 144, and 92% of this cost is a due to labour costs; the latter is cheaper than the mayfly 10 day lethality test but the most expensive of the commercial kits, and 88% of the cost of processing a sample is the cost of the kit itself.

The costs of maintaining cultures for the supply of live organisms for the tests using cultured organisms are presented below in Table 4.7. It should be emphasized that the data in this table reflect both the practices and extent of the culture facilities at UCEWQ. Equipment selection and methods of culturing may vary, and costs incurred by other culture facilities may differ. In addition, the cultures and testing facilities at UCEWQ are capable of supporting tests at rates given in Table 4.3, and changes to the capacity of culture facilities would have further cost implications.

It is apparent from Table 4.7 that the equipment requirements for culturing *D. pulex*, *P. subcapitata*, *S. bicaudatus* and *Chlorella* sp. are far greater than those for cultures of *C. nilotica*. As with the equipment requirements for tests, the greatest part of the cost of equipment for the three expensive cultures is the cost of a water purifier. Apart from this one item, the cost of equipment for the culture of *D. pulex* is R26 721. More specialized equipment is required for the culture of the algae, and costs of equipment for culture of these algae, excluding the cost of a water purifier, are R182 024.

Table 4.7 Overall costs of maintaining cultures of the cultured organisms used in toxicological tests assessed as part of this report. Equipment costs reflect the costs of equipment required to maintain cultures. Consumable and labour costs are expressed on a monthly basis. The total cost per month of culture maintenance is also presented. All cost estimates are based on the protocols of UCEWQ laboratories and assume a similar culture capacity to UCEWQ. All costs are VAT exclusive.

	Start-up cost	**Cost per month**		
	Equipment	**Consumables**	**Labour**	**Total**
Daphnia pulex	R208 189	R254	R1 491	R1 745
Caridina nilotica	R26 472	R318	R2 732	R3 051
Pseudokirchneriella subcapitata	R363 492	R354	R1 076	R1 431
Scenedesmus bicaudatus	R363 492	R354	R1 076	R1 431
Chlorella sp.	R363 492	R354	R1 076	R1 431

The data on culture costs in Table 4.7 can be combined with data on maximal test rates for culture facilities (Table 4.3) to derive the costs per test due to culture maintenance. These are presented in Table 4.8. These estimates are based only on the running costs of culture maintenance, not on equipment costs, and assume that testing is at the maximal rate for these culture facilities. If a higher frequency of testing was required, culture facilities would need to be expanded. If a lower rate of testing was undertaken, the cost per test of culture maintenance would increase as cultures would then not be efficiently utilized. Table 4.8 also presents the full costs of culture-based tests by combining test costs (labour and

consumables from Table 4.5) with culture costs (labour and consumables on a per test basis).

The cost per test of culture maintenance varies considerably between cultures, with algal cultures having the lowest cost on a per test basis, and *C. nilotica* cultures being the most expensive. Although equipment requirements for algal cultures are high, and monthly consumable costs exceed those of cultures of *C. nilotica* and *D. pulex*, labour costs are low and cultures are capable of supporting 20 tests per month (as opposed to 4 tests per month for *C. nilotica* and *D. pulex*). This capacity for supporting a large number of tests is what brings down the cost per test of algal culture maintenance. *Caridina nilotica* cultures require extensive culture facilities and these cannot support as many tests as the algal culture facilities. As a consequence the costs of tests using cultured *C. nilotica* are considerably inflated by culture costs.

Table 4.8 Cost per test of culture maintenance assuming that testing is at the maximum rate of testing for tests that use cultures at UCEWQ laboratories. The full costs per test, combining labour and consumable costs incurred during culturing test organisms for one test and undertaking that test, are also presented. All costs are VAT exclusive.

Culture	Test	Culture cost per test	Full cost per test
Pseudokirchneriella subcapitata	Algal growth inhibition test	R72	R531
Scenedesmus bicaudatus	Algal growth inhibition test	R72	R531
Chlorella sp.	Algal growth inhibition test	R72	R531
Caridina nilotica	*Caridina nilotica* juvenile lethality test	R763	R1 212
	Caridina nilotica 10 day lethality test	R763	R1 401
Daphnia pulex	*Daphnia pulex* lethality test	R436	R888
	Daphnia pulex reproduction test	R436	R2 333

An inspection of full costs per test with all costs, bar equipment, of testing and culturing accounted for, reveals that tests with cultures are generally more expensive than kit-based tests. When costs due to culturing test organisms are not considered, only the *D. pulex* reproduction test stood out as notably more expensive than tests using commercial kits. Once costs due to culturing of test organisms are included, only the algal growth inhibition tests are reasonably cost effective, and these remain cheaper per test than the cost of the similar test using a commercial kit (Algaltoxkit F). The *D. pulex* lethality test is cheaper than two tests using commercial kits (Algaltoxkit F and Rotoxkit F short-chronic), but more expensive than a similar test using a commercial kit (Daphtoxkit F pulex). The *D. pulex* reproduction test and the *C nilotica* 10 day lethality test are both more expensive than any tests using commercial kits.

Potential savings due to shared equipment

One point that arose repeatedly in the basic costing of tests and cultures was that the high cost of a water purifier significantly inflated the equipment costs of several of tests and

cultures. However, one water purifier in a laboratory would suffice for all four tests and listing one water purifier as a cost for each test gives an inflated picture of the cost of equipping a laboratory for several tests. The costs listed above are those that would apply if an organization were to completely equip an empty laboratory for each test. This section of the report assesses the savings that may accrue due to sharing of equipment between tests. Sharing of equipment is likely either when equipping a laboratory to undertake more than one test, or equipping an already extant laboratory to undertake additional tests.

Clearly, there can be no savings due to sharing on costs incurred due to consumables and labour. Only capital equipment can be shared between tests and/or cultures. We initially assessed scenarios where a test using a cultured or collected organism is added to a laboratory already equipped to undertake the other five tests assessed here. This analysis ignored equipment used in culture maintenance. Algal growth inhibition tests are treated as a single test as these share all equipment. We then assessed the costs of adding one culture to a laboratory already equipped for the culture of the remaining two cultured organisms assessed in this report, while ignoring equipment used for the tests. Again, algal cultures are treated as a single culture as all equipment is shared. Finally, the costs of adding all test and cultures simultaneously that use any particular test organism to a laboratory equipped for other cultures and tests is assessed. Once again, algal tests are combined as all equipment for culturing and testing is shared. This approach using groups is taken as much equipment is shared between tests and cultures using the same test organism. A laboratory that is equipped to undertake a test using an organism that is maintained in culture may at minor cost be able to add a second test using that same organism to its repertoire.

For the commercial kits no cultures are maintained, and this section simply assesses where costs can and cannot be reduced as a result of sharing equipment should one test considered here be added to a laboratory already equipped to undertake the remaining five tests.

shows the costs attached to any test, culture, or group of tests and test organisms as outlined above that cannot be reduced by sharing of equipment. These are the costs that would have to be borne on the introduction of a new test or culture or test/culture group to a functioning laboratory. These can be contrasted with the costs presented in Table 4.5 and Table 4.7 to assess savings consequent on equipment sharing.

An examination of the results of the comparison of tests in Table 4.9 indicates by lowered unique equipment costs that tests that share a test organism generally share equipment. The most notable example of this is not explicitly presented in the table: the algal growth inhibition tests share all equipment and therefore laboratory equipped to undertake one algal growth inhibition test could offer another with no extra expenditure on equipment.

Table 4.9 Costs due to unique equipment requirements of tests using cultured or collected organisms compared with other tests using these same organisms, cultures of organisms compared with other cultures, and groups of tests and cultures (where appropriate) using one cultured or collected test organism compared with other test and culture groups. All costs are VAT exclusive.

Tests only (equipment overlap with cultures not considered)		
	Daphnia pulex lethality test	R0
	Daphnia pulex reproduction test	R600
	Mayfly 10 day lethality test	R12 227
	Algal growth inhibition tests	R155 954
	Caridina nilotica juvenile lethality test	R130
	Caridina nilotica 10 day lethality test	R11 105
Cultures only (equipment overlap with tests not considered)		
	Daphnia pulex culture	R13 247
	Caridina nilotica culture	R18 201
	Algal cultures	R168 900
All tests (and cultures) associated with test organism		
	Daphnia pulex culture and associated tests	R4 810
	Caridina nilotica culture and associated tests	R11 657
	Algal cultures and associated tests	R159 699
	Wild-collected mayflies and the mayfly 10 day lethality test	R12 157

The next most notable examples of lowered unique equipment costs owing to sharing of equipment are the two tests that use *D. pulex*. The two tests share all equipment with each other with the sole exception of extra beakers required by the *D. pulex* reproduction test. Here, adding equipment to provide capacity to undertake the *D. pulex* reproduction test to a laboratory already equipped for the *D. pulex* lethality test would cost R600. Clearly, after an outlay of R224 138 for the equipment for the *D. pulex* lethality test, this extra R600 is negligible. The *D. pulex* lethality test could be added to a laboratory equipped for the *D. pulex* reproduction test at no extra cost for equipment.

The other pair of tests that share a test organism are the tests using *C. nilotica*. Inspection of equipment requirements in Appendix A reveal that although the two tests do share most equipment, they also have some unique requirements and they also derive cost savings from equipment shared with other tests. The low unique equipment cost of the *C. nilotica* juvenile lethality test is not therefore entirely due to equipment shared with the *C. nilotica* 10 day lethality test. If the *C. nilotica* juvenile lethality test was added to a laboratory which already was equipped only for the *C. nilotica* 10 day lethality test, a further outlay for equipment of R524 would be required. However, while this is greater than the R130 presented in Table 4.9. it is again a negligible cost in comparison to the full cost of equipment for either test. The *C. nilotica* 10 day lethality test has a much larger unique

equipment requirement. This extra cost is largely due to the requirement for an incubator for this test. Otherwise most, but not all equipment is shared with the *C. nilotica* juvenile lethality test.

The algal growth inhibition tests have by far the largest unshared equipment requirement of all the tests. A large amount of equipment required for these tests, and for the culture of the test species, is not shared with any of the other tests assessed in this report. Most of the savings due to sharing equipment with other tests are accounted for by the sharing a water purification system. It is therefore apparent that the introduction of toxicological testing using cultured algae to an already equipped laboratory will have significant start-up costs.

The mayfly 10 day lethality test does not involve culture maintenance and all equipment costs reflect items used in testing and in collection of test organisms from the wild. Although the artificial stream channels used in this test are not shared with any other test, the savings due to sharing of equipment in the scenarios examined here are nevertheless of an order of 65% of the stand-alone equipment cost. Much of these savings are due to sharing equipment for assessment of water quality and computers for data analysis (both which are used in all tests assessed in this report).

Equipment costs for the culture of all test organisms assessed here are significant. Unlike equipment required for tests, a certain amount of the equipment required for culture maintenance is permanently in use and cannot therefore be shared. The savings that result from maintaining more than one culture in a culture facility are therefore relatively less than the savings found for various tests. In addition, unlike the comparisons between tests presented above, each culture uses a different organism and so equipment requirements are more varied.

As noted above, the major single cost in maintaining *D. pulex* and algal cultures is a water purifier and as this can be shared should both cultures be maintained together, the start-up costs for both cultures are considerably reduced. The water purifier accounts for 87% of the cost of setting up a laboratory for the culture of *D. pulex*. Other potentially shared equipment accounts for a further 6% cost reduction. Of all the cultures assessed here, *D. pulex* culture offers the greatest potential savings due to sharing of equipment with other culture systems.

Equipment for the maintenance of algae in culture are specialized and though significant savings may be realized while culturing algae in the same facilities as the other organisms assessed here, start-up costs of algal culture remain significant even after potentially shared costs are discounted. This is similar to the results of the assessment of potential cost savings due to sharing of equipment for tests. After all possible shared equipment is discounted, the cost of culturing algae in shared facilities remains 46% of the cost of setting up a laboratory from scratch and this cost is far greater than the cost of adding cultures of any of the other organisms assessed here to an equipped laboratory.

When savings due to potential sharing of equipment when adding a culture of *C. nilotica* to an equipped laboratory are examined, it can be seen that the cost of culturing *C. nilotica* in shared facilities is 69% of the total start-up cost for *C. nilotica* cultures. However, costs of starting a culture facility for *C. nilotica* are far less than those of the other organisms assessed, and so, although the savings owing to shared equipment seem relatively low, the absolute cost of establishing a culture facility in an equipped laboratory approximates that of culture facilities for *D. pulex* in the same circumstances.

The above comparisons of tests with other tests, and cultures with other cultures serve to indicate to some extent the savings that may be realized when a laboratory is equipped for more than one test or culture. However, these assessments are also somewhat unrealistic as laboratories undertaking the tests with the organisms assessed above will mostly need to maintain cultures of test organisms. As is apparent in results presented above, tests that share a test organism often share equipment. For this reason, a more realistic idea of the cost of adding tests and their cultures to an extant laboratory can be obtained by looking the cost of adding a culture and the various tests that use that organism. The costs of unshared equipment for all tests and cultures using each test organism assessed here are presented in Table 4.9.

When the results of savings when adding culture/test groups to a laboratory are considered, it can be seen that owing to the at times considerable savings, the costs of adding a new culture and the associated tests to an existing laboratory may be surprisingly low. By far the greatest cost reduction is that noted for cultures of *D. pulex* and the two associated tests. If this group of tests and cultures were to be added to a laboratory equipped for the other tests and cultures considered here, the outlay for the capacity to undertake two extra tests is only R4 810. This is only 2% of the cost of equipping a new laboratory to undertake either test using *D. pulex* (and ignoring costs associated with maintaining cultures).

The most expensive culture and test group assessed here is that of the algal cultures and associated tests. This is, as noted above, largely due to the specialized nature of the equipment required. However, the equipment requirements for the algal cultures and associated tests assessed here could be used to support culturing of a number of algal species as well as running tests using those species following similar procedures.

The mayfly group contains only the costs due to the mayfly 10 day lethality test and no culture-derived costs. As a result, savings when assessing the mayfly test “group” are much the same as those reported for the comparisons using tests alone.

The unshared costs of introducing two tests using *C. nilotica* to a laboratory are much the same as the unshared costs of introducing the *C. nilotica* 10 day lethality test to a laboratory when tests alone are considered. This is approximately 50% or less than the total cost of equipping a laboratory for either test alone or for the culture of this organism.

The costs that would be incurred when introducing a test using a commercial test kit to a laboratory already equipped for tests using the other five test kits assessed here are presented in Table 4.10. As with the analysis of unshared costs in tests using cultured and collected test organisms, it is apparent that considerable savings can be realized through sharing of equipment between tests. Three of the tests share all their equipment with other tests, and the capital cost of adding these to a laboratory equipped for the other tests is therefore R0.

Table 4.10 Costs due to unique equipment requirements of tests using commercial toxicological kits when compared with the equipment requirements of the other five tests that use commercial test kits. All costs are VAT exclusive.

Commercial test kit	
Algaltoxkit F	R5 161
Daphtoxkit F magna	R0
Daphtoxkit F pulex	R0
Rotoxkit F short-chronic	R7 000
Protoxkit F	R0
BioTox	R65 694

The two tests that use *Daphnia* species as test organisms have identical equipment requirements. This accounts for neither test having unshared equipment requirements. However, although some small items of equipment used by this pair of tests are not shared with tests using other test organisms, the majority of the equipment required by these tests is shared with at least two tests using test organisms that are not *Daphnia*. As a result, introduction of both tests to a laboratory equipped for the other kits would realize significant savings.

The test using Protoxkit F also has no unshared equipment requirements. Much of the equipment used for this test is shared with the test using Algaltoxkit F. This applies in particular to the more costly equipment items. The Algaltoxkit F kit's equipment requirements include several standard items of laboratory glassware and a lighted incubator that no other test uses. Nevertheless, the great majority of equipment for this test can be shared with others.

The test using the Rotoxkit F short-chronic kit also has minor unshared equipment requirements as nearly all of the equipment required for this test may be shared.

Of the tests using commercial toxicological test kits assessed here, only the test using the Biotox kit has significant equipment requirements that cannot be shared with other tests. One reason for this is that measurements made during the course of this test assess luminescence rather than optical density or counts of individuals. This necessitates specialized equipment that is not shared with other tests. Nevertheless, sharing of equipment leads to a cost reduction of 43% of the total equipment cost.

Decreasing cost per test as tests are repeated

The costs of introducing a test to a laboratory are strongly affected by equipment costs, especially where sharing of equipment is not possible and expensive and specialized items of equipment are required. This analysis combines start-up equipment costs with the costs of testing to assess overall cost per test with test repetition and to determine how cost per test is reduced as equipment items are re-used. The costs of culture maintenance are included in calculations of overall test cost. No account of cost reductions owing to sharing of equipment between tests is considered here, though sharing of equipment between tests and

associated cultures, if applicable, is accounted for. The algal growth inhibition tests are treated as one due to identical equipment requirements. Table 4.11 shows the overall cost per test at start-up and after equipment is re-used for 100, 1 000 and 10 000 tests. The data presented in Table 4.11 are based on capital equipment costs, costs of labour and consumables for undertaking tests, and, where cultures are maintained, the costs of capital equipment, labour and consumables required for culture maintenance. It is assumed for this analysis that testing is at the rates presented in Table 1.3 for tests that use cultured test organisms. The costs in Table 4.11 will tend to approach the costs of labour and consumables for tests and, where appropriate, for cultures. Data for 10 000 repetitions is presented more to illustrate the extent to which costs can decrease with repetition than in the expectation that laboratories would undertake these tests as many times.

Table 4.11 Overall cost per test of tests as tests are repeated and equipment items re-used. All costs associated with testing and culture maintenance (where appropriate) are used to derive overall test cost. All costs are VAT exclusive.

	Test repetitions			
	1	**100**	**1 000**	**10 000**
Daphnia pulex lethality test	R238 807	R3 267	R1 126	R912
Daphnia pulex reproduction test	R240 852	R4 718	R2 571	R2 357
Mayfly 10 day lethality test	R36 647	R2 489	R2 178	R2 147
Algal growth inhibition tests	R383 358	R4 359	R914	R569
Caridina nilotica juvenile lethality test	R37 194	R1 572	R1 248	R1 216
Caridina nilotica 10 day lethality test	R48 152	R1 868	R1 448	R1 405
Algaltoxkit F	R93 232	R2 167	R1 339	R1 256
Daphtoxkit F magna	R41 228	R975	R609	R573
Daphtoxkit F pulex	R41 145	R892	R526	R489
Rotoxkit F short-chronic	R54 712	R1 532	R1 049	R1 000
Protoxkit F	R100 122	R1 781	R887	R797
BioTox	R116 249	R1 657	R616	R512

The algal growth inhibition tests show the most notable decrease in costs on test repetition. Of all the tests assessed in this report, these have the greatest cost to introduce to a laboratory. However, once tests have been repeated 1 000 times, this test is revealed as one of the cheaper procedures. In comparison to a similar test using a commercial kit, Algaltoxkit F, the algal growth inhibition test using maintained cultures may be cheaper provided that enough tests are undertaken. The cost of the culture-based growth inhibition test and the test using the Algaltoxkit F is roughly equal after 406 tests. Should fewer tests be undertaken, the kit is cheaper; if more, the culture-based test is cheaper.

The mayfly 10 day lethality test is the cheapest of the tests to introduce to a laboratory. However, as a consequence of labour costs during testing and mayfly collection, the cost of

this test does not decrease with repetition as much as any of the other tests assessed here and, after 1 000 repetitions, it is among the most expensive of the tests assessed.

Both of the commercial test kits that use *Daphnia* as a test organism are revealed to be relatively cheap to introduce and to show considerable cost decreases on repetition. When the costs of using the commercial kit using *D. pulex* (Daphtoxkit F pulex) are compared with costs of the *D. pulex* lethality test using maintained cultures, is apparent that the test using the commercial kit is both cheaper to introduce and becomes cheaper still once benefits owing to repetition are considered.

The relatively low costs of the commercial test kits using *Daphnia* are rivalled with repetition only by the costs of the Biotox kit. Although the costs of introducing testing using the Biotox kit are not as high as several other tests using cultured or wild-collected organisms, it is the most expensive of the commercial test kits assessed here. However, costs per test are low and with repetition the costs per test are among the lowest of the tests assessed. Of the tests using cultured test organisms, only the algal growth inhibition test can rival tests using the Daphtoxkit F pulex, Daphtoxkit F magna and Biotox kits for cost efficiency, and then only when a very large number of repetitions are undertaken.

As noted above, the greatest part of the equipment cost of the tests using cultured *D. pulex* is the cost of a single equipment item, *viz*. a water purifier to produce ultrapure water. If this cost is discounted, as it would be if a source of ultrapure water was already available, the costs of these tests are considerably reduced. However, the costs of the *D. pulex* lethality test remain higher than the costs of the similar Daphtoxkit F pulex. This is due to the high cost of culture maintenance, despite a relatively low test cost. If the costs of a water purifier are removed from the *D. pulex* reproduction tests, this remains the most expensive with test repetition of the tests assessed here.

If the costs of a water purifier are discounted from the algal growth inhibition tests, costs are decreased and after 1 000 repetitions, the test, at R732 per test, is the fourth cheapest of the tests assessed.

Of the tests using cultured organisms, the tests using *C. nilotica* are the cheapest to introduce to a laboratory (the mayflies used in the mayfly 10 day lethality test are harvested from the wild and not cultured). These tests remain amongst the cheaper of the tests using indigenous test organisms on a cost per test basis with test repetition. However, many of the tests using commercial test kits become cheaper with repetition than the *C. nilotica* tests. This is largely due to the relatively high costs of *C. nilotica* culture maintenance. Overall, of the tests assessed here, tests using commercial kits have moderate costs on introduction to a laboratory. They are generally cheaper when re-use of capital equipment is considered. Of these, the two kits using *Daphnia* as a test organism are distinctly cheaper after 100 tests, and the same two kits plus the Biotox kit are notably cheaper after 1 000 tests. The most expensive tests after 100 repetitions were the *D. pulex* reproduction test, the algal growth inhibition test, and to a lesser extent the *D. pulex* lethality test. After 1 000 test repetitions the tests that were notably more expensive were the mayfly 10 day lethality test and the *D. pulex* reproduction test.

Of all the analyses of test costs undertaken in this report, the analyses of overall cost per test with repetition is the only one that considers the full cost of culture maintenance as part of the overall culture test. The assumption is made that testing is at the maximal rate

permitted by the culture facilities and therefore that the rate of testing makes most efficient use of the culture facilities. If the rate of testing is below the maximal rate permitted by the culture facilities, the costs of culture maintenance on a per test basis will increase and overall test costs will increase correspondingly. It is therefore important that the rate of testing should be matched to the size of the culture facilities. If testing is sporadic, efficient use of culture facilities will not be possible and this will increase the overall test cost.

4.4 Discussion

Basic test costing and cost of cultures

A simple analysis of start-up costs for test introduction and labour and of consumable costs for testing allows one to rank tests in terms of the costs of introducing tests to a laboratory, and the cost per test once the laboratory is equipped. The results of this analysis indicate that the cheapest test to introduce to a laboratory is the *C. nilotica* juvenile lethality test, and the most expensive are the algal growth inhibition tests. The *C. nilotica* juvenile lethality test is also the cheapest test on a cost per test basis, while the most expensive is the mayfly 10 day lethality test.

This approach does not consider the cost of culture maintenance as part of the overall test cost, or how frequency of testing modifies the overall costs per test, or how re-use of equipment over time or between tests can reduce overall test cost. All of these can considerably modify the cost of undertaking the tests assessed here. A laboratory planning to undertake toxicological testing in a cost-efficient manner would be advised to consider all these factors in selecting tests.

Culture costs

The cost of culture maintenance made up a significant part of the costs of tests using cultures. This applied particularly when culture facilities required significant input in terms of labour or consumables, or when equipment costs were high, and the capacity for supply of test organisms was relatively low. An example here is *C. nilotica*, which requires large culture capacities. The cost of testing a sample using any of the tests assessed here is lower than cost of culturing the shrimp for the test. Algal cultures, though requiring more expensive equipment than the other cultured organisms assessed here, proved relatively cheap to maintain. They are also capable of producing test material for a large number of tests. As a result, the cost per test of algal culture maintenance is relatively low at 16% of the test cost.

When the costs of culture maintenance are factored into test costs, the costs of tests using cultured organisms are increased, to the extent, in some cases, of doubling testing costs. In a simple analysis where costs of testing alone are considered, several of the tests using cultured organisms are more cost-effective than commercial kits. However, when culture costs are considered, most of the culture-based tests assessed were more expensive than kit based tests. In an analysis of the costs of five standard toxicological tests, Persoone and Van de Vel (1988) observed that the costs of culturing test organisms “determined to a substantial extent the ultimate price of a test, especially for acute bioassays”.

When assessing the combined costs of testing and culturing test organisms, an assumption was made that testing was at the maximal rate supported by the culture facilities. If the testing rate was lower than assumed, the cost per test of culturing test organisms would be

greater, and the full cost of tests would increase correspondingly. This indicates the importance of matching culture facilities to test rates in order to minimize culture costs. If testing was infrequent or stochastic, it would be difficult to scale culture facilities appropriately and the cost-effectiveness of culture-based tests would decrease. Persoone and Van de Vel (1988) noted that laboratories that undertook few tests had high costs per test, and that costs per test decreased as the number of tests undertaken increased, largely due to inefficient use of consumables and equipment.

Sharing equipment between tests

Sharing of equipment had a major impact on the cost of introducing a test to a laboratory. If the laboratory in question was already equipped to undertake one or more of the tests considered here (or other, unrelated, procedures), sharing of equipment could reduce or obviate the equipment costs of a test. In our assessment of potential cost reductions due to sharing of equipment, all tests were found to share equipment to some extent and the savings accrued as a result of sharing equipment when introducing a test to a laboratory equipped for other tests ranged from R22 276 to R224 137, or 43% to 100% of the cost of the test being introduced.

The two tests using cultured *D. pulex* that are assessed in this report are both tests mandated under DEEEP (Slabbert, 2004). They share nearly all equipment and the costs of implementing both tests in a laboratory (excluding running costs for testing and culture maintenance) are roughly the same as implementing one test. In all tests using cultured test organisms, the costs of introducing a second test using the same test organism (or, in the case of the algal growth inhibition tests, a different taxon where culturing and testing followed roughly the same protocol) were considerably reduced due to sharing of test equipment and culture facilities.

Tests using commercial test kits, particularly those sourced from MicroBioTests Inc, shared most or all of their equipment with other kits, with the consequence that a laboratory equipped for one test could generally undertake others with minor or no equipment purchases. This seems to largely be a function of assessing several tests from MicroBioTests, Inc. A number of other commercial toxicity test kits are available from a range of manufacturers (for examples see Blaise, 1998; Mitchell et al., 2002; Daniel et al., 2004; Wadhia and Clive Thompson, 2007). Some of these have sophisticated equipment requirements and could not share equipment with tests assessed here.

The cost analyses presented here examined a scenario where a group of tests using cultured and wild-collected organisms were assessed together for sharing of equipment, and another where a second group of tests using commercial kits were assessed together. A scenario where a combination of tests using cultured and collected organisms and tests using commercial kits were assessed together to determine equipment overlaps was not undertaken. Equipment requirements largely differed between the groups.

All analyses of sharing of equipment compared the equipment requirements of one particular test with those of the remaining five tests using either commercial test kits or cultured and collected test organisms. The considerable overlap of equipment requirements noted is to some extent a function of the comparison of one test with a relatively large group of tests. If one test is compared with only one other, the potential for sharing equipment may reduce.

Nevertheless, certain tests, for example the tests using cultured *D. pulex,* and the commercial kits using *D. pulex* and *D. magna*, shared nearly all equipment.

Start-up costs of test introduction were often largely due to the costs of one or few expensive equipment items. The most notable of these was a water purifier for three tests that require ultrapure water, which made up 48-81% of equipment costs. As one purifier would be sufficient for all three tests, the costs of the purifier could be shared between the tests and the consequent costs of test introduction would, on a per test basis, be reduced. Alternately, depending on the amount of ultrapure water needed, it might be more cost effective for a laboratory to purchase ultrapure water from another source rather than to install a purifier. This would particularly be the case when relatively small quantities of water were required. Some other equipment items, particularly those used in culture maintenance, could also be substituted for, and this would need to be addressed on a case-by-case basis.

Test repetition and re-use of equipment

Should a laboratory choose to undertake testing using either commercial kits or indigenous organisms, it will initially need to purchase sufficient equipment to undertake testing and maintain cultures. The upfront costs of equipping a laboratory for testing, ignoring sharing of equipment, are high and range from R23 565 to R378 604 for testing alone. However, equipment can be re-used and, in assessing the contribution of upfront equipment costs to the costs of tests, we assessed how overall test costs changed depending on how frequently the test was undertaken. This approach was chosen so as to compare changes in overall test costs between tests with high initial costs but low ongoing costs, such as the algal growth inhibition tests, and tests with low initial costs but a high cost per test, such as the mayfly 10 day lethality test.

Clearly, tests with lower initial costs and low costs per test should remain cost-effective regardless of test repetition. However, tests with higher initial costs and low costs per test may, given enough repetitions, become more cost effective than tests with lower initial costs but a higher cost per test. The best examples to illustrate this are the algal growth inhibition tests, which have the highest equipment costs of any of the tests assessed, but the third lowest cost per test inclusive of culture costs. Although when few repetitions are considered the algal growth inhibition tests are the most expensive of the tests assessed, after 80 tests the algal growth inhibition tests have roughly the same cost per test as the *D. pulex* reproduction test. At maximal test rates for the algal growth inhibition tests, this corresponds to four months of testing. The algal growth inhibition tests reach cost parity with the mayfly 10 day lethality test after 216 repetitions, or approximately 11 months of testing. With an increasing number of repetitions, the algal growth inhibition tests become more cost effective than most tests assessed here. They reach cost parity with a similar test using the commercial kit Algaltoxkit F after 406 repetitions, or less than two years of testing at the maximal rate.

From the above it is apparent that laboratories need to consider the number of tests they anticipate undertaking before selecting tests based on test cost. For a laboratory anticipating undertaking 80 or fewer tests, the algal growth inhibition test is the most expensive of the tests considered here. If 1 000 tests were to be undertaken, it is one of the cheapest.

As noted above, if a test has low initial equipment costs and a low costs per test, it would remain cost-effective regardless of the number of times a test would be repeated. Examples here are the tests using commercial kits Daphtoxkit F pulex and Daphtoxkit F magna, which are used to undertake a very similar test on *D. pulex* and *D. magna* respectively. These tests are amongst the most cost-effective regardless of the number of tests undertaken.

The cost per test of any test cannot decrease below a limit set by the cost of labour and consumables required for testing together with, where appropriate, the cost of labour and consumables using in culture maintenance. Of the tests assessed here using cultured indigenous organisms, the costs of producing test organisms for testing ranges from 16-170% of the cost of undertaking the test. At the lower end of this range, the algal growth inhibition test has a relatively low cost of producing test organisms, and this test may be cost-effective, despite high initial costs, given enough repetitions. At the upper end of the range, tests that would have been competitive if the testing cost alone had been considered are rendered costly as a result of culture costs. All the tests using *C. nilotica* as a test organism fall into this category.

Outsourcing and DEEEP tests

As an alternative to undertaking tests themselves, an organization may choose to outsource testing to an external laboratory. Of the toxicological tests using cultured test organisms assessed in this report, three are recommended under DEEEP: the *D. pulex* lethality test, the *D. pulex* reproduction test, and the algal (*P. subcapitata*) growth inhibition test. Tests using commercial test kits undertake two matching tests using the Daphtoxkit F pulex and the Algaltoxkit F.

Should relatively few tests be required, it will always be more cost-efficient to outsource tests to an external laboratory, as equipping a laboratory for testing incurs equipment costs that cannot be justified for a few tests. We have used data from the costing of tests excluding equipment costs, and data assessing how the full cost per test drops with repetition, to compare the test costs as assessed here with the quotes we obtained from commercial laboratories.

The algal growth inhibition test can be undertaken with labour and consumable costs of R531 and R1 247 for the culture-based test and the commercial kit, respectively. Both of these fall below the range of costs quoted by external laboratories for this test. When equipment costs are included in the overall test cost and cost recovery on repetition is considered, the culture-based test is cheaper than the cheapest of the quotes from external laboratories after 370 tests, and the kit-based test is cheaper than the cheapest quote after 289 repetitions. This comparison assumes that all equipment required for the tests was purchased for that purpose.

If some equipment was available from elsewhere, or shared, fewer tests would be necessary to bring culture or kit-based tests to cost parity with the cheapest quote (for example, if an alternate source of ultrapure water were available, the culture-based test would be cheaper after 195 tests). If all equipment were to be purchased for this test, outsourcing would be cheaper should fewer than 289 tests be planned. Should 289-405 tests be required, testing using the Algaltoxkit F would be most cost-efficient, and, should 406 or more tests be envisaged, testing using a culture-based test would be most cost-efficient.

The *D. pulex* lethality test can also be undertaken using a culture or kit-based test, with labour and consumable costs of R888 and R485, respectively. These are both less than the cheapest of the quotes from external laboratories. When all costs due to equipment, without consideration of savings due to sharing of equipment or alternate sources of equipment, are included in this comparison, the culture-based test is more cost efficient after 981 tests, and the test using the Daphtoxkit F pulex kit is cheaper than quoted prices after 94 tests. As the test using the commercial kit is always more cost efficient than the culture-based test in this analysis, one may conclude that outsourcing of testing is cost-efficient when 93 or fewer tests are planned. Should more tests be planned, testing using the Daphtoxkit F pulex kit is the most cost-efficient approach to undertaking the *D. pulex* lethality test.

The *D. pulex* reproduction test is the most expensive of the tests for which we have quotes from external laboratories. The costs of undertaking this test using cultured *D. pulex* were determined; however, no commercial kit is available for comparison. The combined labour and consumable costs of undertaking this test using cultured *D. pulex* is R2 333, which is less than half the cost of the cheapest of the external quotes. When equipment costs are factored in and equipment cost recovery with test repetition considered, the culture-based test is found to be more cost- efficient than outsourcing the test after 70 tests. Savings due to sharing of equipment are not considered in deriving this figure and should such savings be possible, the culture-based test would be more cost efficient than outsourcing after fewer than 70 tests (for example, an alternate source of ultrapure water would make the test using cultured *D. pulex* the cheaper approach after only 17 tests). Should an organization envisage undertaking this test less than 70 times, it would be more cost-efficient to outsource the testing should all equipment for undertaking the test in-house needed to be purchased. If the test is required 70 times or more, equipping a laboratory to undertake the test using cultures would be more cost-efficient.

General recommendations

The analyses presented here indicate the importance for a laboratory intending to institute toxicological testing of careful consideration of what they plan on testing and how they institute testing. Issues relating to ecological suitability and defensibility of tests need consideration and these are addressed elsewhere in this report. Here, we will only address general issues pertaining to cost.

The cost analyses presented here are based on the practices of two laboratories and results reported on will not apply exactly to all other laboratories. Issues such as equipment selection and methods of culturing may vary between laboratories and these will influence the costs of testing. Persoone and Van de Vel (1988) found that costs varied considerably between laboratories, and were influenced by the type of laboratory (commercial, research, *et cetera*), amount of testing undertaken, and laboratory practices (good laboratory practice reduced costs by 30%). Regardless of a degree of differences between laboratories, the general trends affecting costs that were identified here should apply in all circumstances.

Equipment costs varied considerably between tests. Test that used a minimum of specialized equipment, for example the mayfly 10 day lethality test and tests that used *C. nilotica* as a test organism, had particularly low start-up costs. Cost of equipment for tests using commercial test kits were in some cases low, and were never as high as the costs of the more equipment-intensive tests using indigenous organisms. One factor here is that

equipment for kits was generally sourced from kit producers and was designed for low cost and robust use (MicroBioTests Inc, 2009). Similar equipment sourced from a general scientific supplier would in most cases be more expensive.

Costs of equipment used in tests were often high. These costs will arise whether a laboratory undertakes one test or many. We have presented several scenarios where re-use of equipment over time or between tests results in considerable cost reductions on a cost per test basis. However, no single prescription on a particular battery of tests will suit all users. Laboratories will need to assess what equipment they have, what they need, and what their testing requirements are before deciding on tests that would be most cost effective for them. If tests using cultured organisms are considered, multiple tests on a single test organism will allow some rationalization by minimizing the number of cultures maintained. The scenarios explored in this analysis do not cover all possible combinations of tests, but indicate certain trends and possibilities for reducing equipment costs in testing.

Costs relating to labour and consumables cannot be reduced through planning of tests in the same way that equipment costs may be. The combined cost of labour and consumables for testing, and for culture maintenance where appropriate, is the absolute minimal cost of undertaking any test and, should equipment costs be reduced to zero, for example by sharing of equipment, labour and consumable costs would define the cost of the test. Commercial kits generally had low labour requirements but higher consumable costs. The consumable costs for commercial kits largely reflect the cost of the kits themselves, as the kits contain most of the consumables required. In contrast, consumable requirements for the tests using indigenous organisms are low, and test costs tend to reflect the labour requirements of the tests and of culture maintenance. These more labour-intensive tests also mean lower test throughput.

All tests using cultured organisms incurred costs owing to culture maintenance. Of these, only costs of culturing algae were relatively small compared to the test cost, and then only provided that rate of testing is high. In the remaining cultured test organisms assessed, the costs of culturing were high and impacted negatively on test cost-efficiency.

A consideration of the costs of testing in the light of examples of the cost of outsourcing tests reveals that all tests assessed here for which external quotes for testing were available can be undertaken more cheaply in-house than at the quoted prices. However, the difference between the quoted prices and the costs of undertaking the test using indigenous organisms or commercial kits varied from test to test. The cost-efficiency of undertaking in-house testing, rather than outsourcing tests, needs to be determined for each test as each has a threshold number of tests below which it proves cheaper to outsource testing.

The results of this cost analysis are broadly indicative of trends and individual laboratories would need to assess their own testing requirements on a case-by-case basis. For example, depending on availability of skilled labour and availability of equipment in laboratories, it may be appropriate to weight these differently during test selection. Should equipment and consumables be available or easily accessible, but skilled labour scarce or unavailable, it may be better to choose tests where labour costs are a relatively small part of the overall test cost. In this case, the tests selected would generally be tests using commercial kits, as these have been devised to be easy and rapid to use. The cost of consumables when using kits is relatively high, but users are spared the burden of culture maintenance with its accompanying demands for labour input. A laboratory having access to skilled labour and

general laboratory equipment may find the tests using cultured organisms more suitable as costs due to consumables are generally low.

5 GENERAL DISCUSSION

5.1 Outsourcing of testing

Outsourcing of toxicological testing has considerable advantages in that tests can be undertaken by trained staff in accredited laboratories thereby producing defensible results with standardized methods. Organizations choosing to outsource testing will also not have to train or attract skilled staff to undertake tests, or to source space and equipment for a laboratory, and possibly for culture facilities.

The cost implications of outsourcing, however, make this the most expensive approach to testing when costs of consumables and labour of testing alone are considered. When capital costs of equipment are considered, outsourcing is seen to be the cheaper option when fewer tests are required. For assays using *Daphnia pulex*, outsourcing becomes less cost effective after 70-100 tests. The algal (*Pseudokirchneriella subcapitata*) growth inhibition test was cheaper to outsource when undertaking less than 200-400 tests, depending on laboratory configuration.

If outsourcing is selected, organizations will also need to locate a commercial laboratory that is able to undertake the required test(s) when required and within an appropriate time. As no laboratory willing to undertake the Ames *Salmonella* test was found during this project, this may not be a trivial issue.

5.2 In-house testing

DEEEP tests using cultured test organisms and commercial kits

Of the tests assessed here, two of the three standard DEEEP culture-based tests (the *D. pulex* lethality test and the *P. subcapitata* growth inhibition test) performed no better or worse than matching tests using kits. The remaining DEEEP culture-based test, the *D. pulex* reproduction test, produced no results owing to difficulties in producing neonates. None of these tests produced explicit endpoints for more than one effluent. Derivation of endpoints was complicated by high levels of toxicity in one effluent, colour casts in two effluents interfering with effect assessment, and growth stimulation in algal tests caused by two of the effluents.

The *Vibrio fischeri* bioluminescence test returned two explicit endpoints, and in terms of ease of use was one of the more tractable tests considered. However, this test was one of the least sensitive of those considered for this report. It also showed increased bioluminescence in one of the effluents.

The cost of undertaking the *D. pulex* lethality test using standard methods and kits is comparable only if culture costs are not considered. When the costs of producing test organisms is added to the cost of undertaking the assay, the standard test is significantly more expensive than the kit. On first inspection, the cost of equipping a laboratory to undertake the standard test is greater than equipping a laboratory for testing using a kit. However, this comparison is based on the practices of two laboratories and is affected by the equipment selected by each laboratory. If the two tests use the same source of ultrapure water, the equipment costs are comparable.

The cost of undertaking the *P. subcapitata* growth inhibition test using a kit is considerably more than the cost of using the standard method, even when culture costs are considered. However, costs of equipping a laboratory for testing using the standard method are much greater than costs of equipping a laboratory for testing using kits, even when differing laboratory practices are considered. For this reason, use of the standard method is more cost effective only when a high throughput of tests is anticipated.

The cost of undertaking the *D. pulex* reproduction test using standard methods is high, and, when culture costs are considered, this is the most expensive of all the tests assessed in this report. Costs of equipment for this test are also high, although most of this is the cost of a single item and, if another source of ultrapure water is found, equipment costs are reasonable. It is worth noting that the *D. pulex* reproduction test shares nearly all equipment with the *D. pulex* lethality test using the standard method, and a laboratory equipped for one could undertake the other test with little or no extra expenditure on equipment.

The *V. fischeri* bioluminescence test using a kit was amongst the most cost-effect of the tests assessed. Although equipment costs are relatively high, owing to the low cost of undertaking the test, after 100 test repetitions the overall cost per test is amongst the lowest of the tests considered.

The use of toxicological tests using standardized methods with test organisms drawn from laboratory culture has a long history and many of the methods used have been well validated (e.g. see Römbke et al., 2009). Given this background, these standard tests would seem the obvious choice for instituting routine toxicological testing. In addition, the Department of Water Affairs favour standard methods owing to their suitability for legislation and routine monitoring (DWAF, 2003).

Standard culture-based tests have been criticized on several bases, however. The cost of maintaining cultures can add considerably to the test cost even in well-managed facilities (Persoone and Van de Vel, 1999). The results of this project concur and show that culture costs accounted for up to 63% of test costs. Culture costs varied depending on the test organism, with algal cultures offering the lowest cost per test of the tests assessed here.

The low culture cost per test for algal tests is based on maximal throughput for testing without expanding the culture facility or staff. If lower rates of testing occurred, the cost per test of maintaining cultures would increase. This generality applies to all tests that use test organisms from laboratory culture. Increasing the rate of assay throughput beyond the maximum rate considered here would require expanding the culture facility, which would have cost implications (though possibly a lowered overall cost per test owing to economies of scale). It is therefore important that the rate of testing is matched to the size of culture facilities, and that, in order to assure efficient use of cultures, the rate of testing should be constant and predictable. This may best be achieved in laboratories that process routine samples where the rate of testing is largely known in advance. Commercial toxicity test kits do not require cultures and so are better suited to use when testing is irregular.

Another issue with throughput identified as a drawback of standard culture-based test methods as opposed to toxicity test kits is the potential of the latter for a greater test throughput (Mitchell et al., 2002). In the assays assessed during this report, the amount of time, as labour and not reflecting the time each assay ran for, required for running the same

test using kits was less than that for culture-based methods. This may limit the potential throughput of tests using standard methods.

Tests using indigenous test taxa

The *Caridina nilotica* juvenile lethality test returned more explicit endpoints than any other test assessed here and was generally sensitive, especially to the less toxic organically enriched effluents. Although the cost of undertaking this test is fairly low, as is the cost of equipping a laboratory for the test, culture costs are significant and make this one of the more expensive tests overall. Nevertheless, in light of the ease of application of the test, its sensitivity in response to the effluents assessed here, and its ecological relevance, this test deserves consideration for wider application in South Africa.

In contrast, the *C. nilotica* 10 day lethality test returned no valid endpoints. However, this test was run using diluted effluent based on the results of the *C. nilotica* juvenile lethality test, and the range of dilutions selected was too high for endpoint derivation. Unfortunately, insufficient effluent was available for test repetition. Further research is required to assess this test's efficacy.

Use of the mayfly 10 day lethality test was complicated by the requirement for very large quantities of effluent for testing. This precluded test repetition and raised issues relating to disposal of large quantities of toxic effluent following testing. Despite relatively low equipment requirements, the test requires much time with consequent labour costs, resulting in this being one of the more expensive of the tests assessed here. As this test used test organisms from a wild population in the region that the test effluents are discharged, it has high ecological relevance. When this test was run using dairy and textile effluent, mayflies from the families Tricorythidae and Leptophlebiidae respectively showed limited mortality even at high effluent concentrations. It seems from this that these families may be less sensitive to these effluents than several other tests assessed. In addition, neither family showed a clear dose response to wastewater treatment works effluent. Only tannery effluent evinced a clear dose response from both families, though, as the test could not be repeated, precise endpoints were not generated.

The cost implications of applying the algal growth inhibition tests using indigenous algae were identical to those of the standard *P. subcapitata* growth inhibition test. All of these tests are expensive to introduce, yet, when the tests are undertaken sufficient times, the cost per test becomes competitive. In addition, a laboratory that is equipped for any one of the algal growth inhibition tests assessed here could introduce the others at no extra capital cost. As these tests use indigenous taxa, their ecological relevance is high. Application of these tests to the effluents assessed during this report was complicated by the colour cast of two of the effluents leading to testing in diluted effluent only. The remaining effluents all resulted in increased growth (with the sole exception of *Scenedesmus bicaudatus* in wastewater treatment works effluent) and therefore no toxicological endpoints could be derived for these effluents. As a result it is difficult to comment on the sensitivity of these tests using the results presented here and further research is necessary to properly evaluate these indigenous algal growth inhibition tests.

Other toxicity test kits

The *Brachionus calyciflorus* reproduction inhibition test and the *D. pulex* reproduction test are the two tests assessed in this report that examine inhibition of reproduction, rather than mortality or growth inhibition as in the majority of other tests assessed. The *B. calyciflorus* reproduction inhibition test returned one precise endpoint in wastewater treatment works effluent, while in the remainder of the tests mortality owing to the effluents was too high to validly determine reproduction inhibition. This suggests that this test may be one of the more sensitive of those assessed, a conclusion supported by other test comparisons (Römbke et al., 2009). The costs of this test are roughly mid-range among the tests assessed, and equipment costs are relatively low. As *B. calyciflorus* has been reported from South African waters (Jarvis et al., 1987; Brain et al., 1995), this test has ecological relevance for application in South Africa. As this test is cheaper than the *D. pulex* reproduction test, and, as shown in this study, was easier to apply, and as it appears sensitive and also has ecological relevance, it would appear worth a closer assessment to determine whether it might be appropriate for use in routine application in South Africa.

Daphnia magna is widely used internationally as a test taxon for toxicological testing, as to a lesser extent is *D. pulex* (Mitchell et al., 2002). Tests using *D. pulex* were selected for routine application under DEEEP (Slabbert, 2004). The endpoints returned by the *D. magna* lethality test here do not differ significantly from those of the *D. pulex* lethality test (standard method or test kit). The *D. magna* lethality test using the Daphtoxkit F magna is more expensive than the *D. pulex* lethality test using the Daphtoxkit F pulex, though cheaper than the *D. pulex* lethality test when following the standard culture-based methods. Given the ecological relevance of *D. pulex* in South Africa and considering the results reported here, there seems no reason to recommend routine application of the *D. magna* lethality test either together with or in the place of the *D. pulex* lethality test.

The *Tetrahymena thermophila* growth inhibition test was one of the less successful of the tests assessed here in terms of returning endpoints to tests. One of the reasons for this relates to interference by the two coloured effluents as the test uses changes in optical density of a food substrate that is consumed by the ciliates. However, this test did not show a dose-related response in the non-coloured effluents. The test cost is moderate, while equipment costs are fairly high. Given the difficultly experienced in deriving endpoints for a range of effluents and the low ecological relevance of this taxon for South Africa, this test is considered one of the less promising of those assessed.

5.3 Laboratory configuration and choice of tests

In equipping or expanding a laboratory undertaking toxicological testing, rationalization, where possible, of test choice can lead to considerable savings on capital equipment costs largely through sharing of equipment between tests. Many of the tests assessed here share most or all of their equipment with other tests, thereby reducing the need for equipment purchases when undertaking several tests together at the same facility. Rationalization will depend on the goals of the facility in question, and these will differ between, for example a laboratory offering commercial testing with a number of commonly applied tests and an in-house laboratory undertaking only those tests mandated for the organization in question.

Selection of equipment will have implications for cost, ease of use and throughput of samples. Simpler, manual equipment will generally be cheaper to purchase, while the use of

more expensive items that increase throughput either through more effective design or by offering some degree of automation may decrease sample handling time and increase test throughput. The choice will depend on the goals of the laboratory and in particular on anticipated sample load.

In a similar light, cost of equipment on a per test basis is reduced when equipment items are reused. If the number of tests to be undertaken is likely to be low, then equipment costs make up a larger portion of the test cost, and purchase of simpler and cheaper equipment may be justified. When the number of tests anticipated is greater, purchase of more expensive equipment that might streamline sample processing would be justified as the increased cost, expressed on a per test basis, is minor.

5.4 Issues encountered and further research

Two of the complex effluents assessed in this study were coloured, and attempts to decolourize these achieved little. Several of the toxicological tests employed rely on measurements of optical density to assess effect levels, and increases in optical density of the test solution as a result of effluent colour complicate this measurement. Coloured effluents may also interfere with light penetration and consequent photosynthesis in tests using algae as test organisms (Cleuvers et al., 2002). Several other tests relied on counts of test organisms which were difficult to undertake in coloured or turbid effluents. Attempts to circumvent this problem by running tests on highly diluted effluent were not always successful.

It has been proposed that, when dealing with coloured or turbid effluents, algal growth inhibition tests be replaced with another test, using a vascular plant as a test organism, where growth and effect measurement are not affected by the opacity of the test solution. *Lemna* species are most commonly used in this regard (Cleuvers and Ratte, 2002; Chandra and Singh, 2005; Römbke et al., 2009). Authors differ as to the relative sensitivity of the *Lemna* test in comparison to the algal growth inhibition test, with some reporting the *Lemna* test as less sensitive than the algal test (Römbke et al., 2009), others finding the *Lemna* test more sensitive (Cleuvers and Ratte, 2002), and others undecided (Keddy et al., 1995). One drawback to use of the *Lemna* test is that it takes 7 days to complete and so is considerably slower than the algal growth inhibition test. A faster whole plant bioassay that may be worth consideration is a similar bioassay using *Spirodela* spp. (e.g. see Oberholster et al., 2009). Following international practice and in the light of experience in this study, we strongly recommend that a test be included in DEEEP as an alternative to the algal growth inhibition test and/or other tests that measure changes in optical density when coloured effluents are to be tested. Depending on ongoing experience, it may be necessary as well to consider alternative tests where coloured or turbid effluents preclude accurate counting of other test organisms.

The issue of test repetition after initial testing failed for some reason to produce valid results arose several times during this study. Occasional repetition of testing in this way is an ongoing issue in laboratories undertaking routine toxicological testing. Test repetition raises issues relating to sampling and in particular sample storage. For the majority of tests mandated under DEEEP, samples are collected and stored at 4ºC in the dark, and testing initiated within 24-36 hours of sampling (Slabbert, 2004). As the majority of tests assessed in this report take longer than this to complete, it may not be known whether tests need to be repeated until the sample storage time has been exceeded. As a result, collection of

duplicate samples as a provision for test repetition is not possible. This may be less of an issue when sampling can easily be repeated, but where this is not the case then testing would need to be aborted. As freezing of samples is explicitly excluded under DEEEP (Slabbert, 2004), no obvious solution to the problems posed by the possibility of test repetition is apparent.

Several of the effluents assessed during this study caused effect stimulation in certain of the assays. The algal growth inhibition tests in particular were affected in this way, as well as, in one case, the *V. fischeri* bioluminescence test. Increased bioluminescence in the latter test has been noted before (e.g. Cook et al., 2000; Hemming et al., 2002). Growth stimulation in the algal tests was likely due to nutrient enrichment in the effluents. These results do not indicate flaws in the tests themselves but underline the requirement for testing using a range of tests with varying test taxa.

This report assesses the results and cost of toxicological testing using a range of whole-organism tests that focus on lethal (acute) and sublethal (chronic) toxicity. Measurements of oxygen demand were also taken. Beyond these, DEEEP also proposes assessment of effluent potential for bioaccumulation, mutagenicity and persistence (DWAF, 2003). The tests assessed here represent the traditional approaches to ecological risk assessment (Mitchell et al., 2002). A range of other approaches exist. One is the assessment of toxic effects at the cellular, subcellular and biochemical level, which may provide an advance warning of toxicant action (Adams and Tremblay, 2003). Another approach examines the toxic effects of effluents at the community and ecosystem level, in the process revealing the impact of indirect effects that are masked in simple assessments of a few target taxa (Preston, 2002). All of these approaches deserve serious consideration as tools for effective management of ecosystems in the face of effluent disposal into water bodies.

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APPENDIX A: EQUIPMENT AND CONSUMABLES USED IN THE VARIOUS TESTS

This appendix contains full lists of equipment and consumables used in all tests and cultures that are assessed in this report. Tables show the number or quantity of items required and the matching cost. Also listed are costs due to time spent in activities associated with tests. All estimates follow the practices of the laboratories of the Unilever Centre for Environmental Water Quality (UCEWQ) for standard tests using cultured and wild-collected organisms, and those of Toxsolutions Kits and Services for test using commercial kits.

Tables with equipment requirements list the items and costs of equipment required in order to undertake a particular test (Table A.1, Table A.2). These are required before a test can be undertaken. Tables with consumable requirements list the quantity and cost of items used in one of the assessed tests to process one effluent sample (Table A.4, Table A.5). Tables with time requirements show the amount of time required to undertake various tasks associated with testing (Table A.7, Table A.8) Tables with equipment requirements for cultures reflect the equipment requirements to maintain cultures at the same level as at UCEWQ (Table A.3). Tables with consumable requirements for cultures list the quantity and cost of items required to maintain cultures, again at the same level as UCEWQ, for one month (Table A.6). Time required to maintain cultures for one month at the level that they are maintained at UQEWQ and the associated cost is also presented (Table A.9).

The total cost of setting up a laboratory from scratch to run any test or maintain any culture is listed at the bottom of the equipment tables. Likewise, the total cost of consumables used in testing one sample or maintaining any culture for one month is given at the bottom of the appropriate tables. These overall costs are the same as those listed in the body of the report. Minor differences are due only to errors resulting from rounding of long decimals.

Certain of the units given may appear unusual at first glance. For example, the cost of water treatment is presented as the cost per litre of treated water, while the line item is often a filter or cartridge. In this case, the cost presented is the cost of replacing the cartridge or filter and is expressed as a fraction of the lifespan, in litres, of that cartridge. Cases such as this where some confusion might arise are directly addressed in the table captions.

The costs of equipment and consumables are based on quotes from suppliers obtained during 2008. All costs are VAT exclusive. All labour costs are calculated based on Rhodes University's pay scales.

Table A.1 Quantity and cost in Rands of equipment required to undertake the toxicological tests using indigenous organisms that are assessed in this report. Equipment listed here is that used in the laboratories of UCEWQ. Software is listed as having no cost as open-source software is available that is suitable for the requirements of the tests. All costs are VAT exclusive.

	***Daphnia pulex* lethality test**		***Daphnia pulex* reproduction test**		**Mayfly 10 day lethality test**		**Algal growth inhibition test**		***Caridina nilotica* juvenile lethality test**		***Caridina nilotica* 10 day lethality test**	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Aeration stones					10	14.30			2	2.86	2	2.86
Air pumps for fish tank									1	46.75	1	46.75
Air pumps for fish tank (portable, battery powered)					10	399.10						
Air tubing 5mm (25 m roll)					1	22.23			0.08	1.78	0.08	1.78
Autoclave							1	25 422.00				
Balance, laboratory, coarse	1	2 109.00	1	2 109.00			1	2 109.00				
Balance, laboratory, fine	1	11 086.50	1	11 086.50			1	11 086.50				
Beakers, glass (50 mℓ)	60	600.00	120	1 200.00			5	50.00				
Beakers, glass (100 mℓ)	5	50.00	5	50.00								
Beakers, glass (500 mℓ)	2	41.46	2	41.46			5	103.65				
Beakers, glass (600 mℓ)									16	416.00	35	910.00
Beakers, glass (1000 mℓ)							5	135.00				

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Beakers, glass (3000 mℓ)	2	350.00	2	350.00								
Container for collection of mayflies					5	657.90						
Buckets (plastic, 25 ℓ)					20	630.80						
Bunsen burner							1	186.00				
Computer	1	6 000.00	1	6 000.00	1	6 000.00	1	6 000.00	1	6 000.00	1	6 000.00
Conductivity meter	1	3 329.00	1	3 329.00	1	3 329.00	1	3 329.00	1	3 329.00	1	3 329.00
Conductivity meter probe	1	738.00	1	738.00	1	738.00	1	738.00	1	738.00	1	738.00
Control valves for air tubing									2	2.10	2	2.10
Dissolved oxygen meter and probe	1	5 394.00	1	5 394.00	1	5 394.00	1	5 394.00	1	5 394.00	1	5 394.00
Deionising column for water	1	3 923.00	1	3 923.00			1	3 923.00				
Forceps					5	285.00	1	57.00				
Gas cylinder (LPG, 9 kg)							1	152.63				
Graduated pipettes, glass (1 mℓ)							10	90.00				
Graduated pipettes, glass (5 mℓ)							10	100.00			5	50.00

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Graduated pipettes, glass (10 mℓ)							20	240.00			5	60.00
Graduated pipettes, glass (25 mℓ)							10	190.00				
Haemocytometer							1	355.68				
Heater/magnetic stirrer	1	2 400.00	1	2 400.00			1	2 400.00				
Ice bricks					10	131.60						
Incubator											1	10 611.12
Incubator (lighted)							1	17 800.00				
Light box (overhead light source)	1	200.00	1	200.00					1	200.00		
Magnifying glass	1	68.40	1	68.40					1	68.40	1	68.40
Measuring cylinder (50 mℓ)	2	52.20	2	52.20			2	52.20				
Measuring cylinder (100 mℓ)	2	62.00	2	62.00			3	93.00				
Measuring cylinder (250 mℓ)	8	472.00	8	472.00								
Measuring cylinder (500 mℓ)							2	156.50			1	78.25
Measuring cylinder (1000 mℓ)					1	125.00	1	125.00	2	250.00		

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Mesh strips, nylon (1 m × 10 cm)					20	2 238.40						
Micropipette (10-100 µℓ)							1	899.00				
Micropipette (100-1000 µℓ)	1	799.00	1	799.00			1	799.00				
Microplate reader (24 well plates)							1	42 000.00				
Microplate reader software							1	8 508.77				
Microscope, compound							1	50 085.00				
MilliQ water purification system	1	181 468.00	1	181 468.00			1	181 468.00				
Net (fine nylon mesh)									1	5.50	1	5.50
Paintbrush (fine-medium, soft)					5	17.55						
pH meter	1	3 365.00	1	3 365.00	1	3 365.00	1	3 365.00	1	3 365.00	1	3 365.00
pH meter probe	1	633.00	1	633.00	1	633.00	1	633.00	1	633.00	1	633.00
Photosynthetic photon flux density (PPFD) meter							1	3 580.00				
Pipette bulb for pasteur pipette	4	36.00	4	36.00					1	9.00	1	9.00
Pipette bulb/pipette filler							1	89.00	1	89.00	1	89.00

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Pipette canister							1	388.00				
Pipettes, glass (10 mℓ)	2	24.00	2	24.00					2	24.00		
Pipettes, glass (25 mℓ)	5	95.00	5	95.00								
Plastic bowl (2 ℓ)									2	26.32	2	26.32
Plastic sheet	1	1.00	1	1.00								
Plastic tubing, Tygon (1 m)					30	4 410.00						
Reagent bottles, glass (100 mℓ)	2	50.00	2	50.00			2	50.00				
Refrigerator (4°C)							1	2 833.33				
Sample bottle, polyethylene (100 mℓ)	1	8.00	1	8.00			1	8.00				
Sample bottle, polyethylene (500 mℓ)	4	36.00	4	36.00								
Schott bottles (100 mℓ)							5	78.00				
Schott bottles (250 mℓ)							10	169.20				
Schott bottles (1000 mℓ)							10	256.50				
Software, spreadsheet	1	0.00	1	0.00			1	0.00	1	0.00	1	0.00
Software, statistical	1	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1	0.00

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Spatula	1	140.00	1	140.00			2	280.00				
Stirrer magnets	2	60.00	2	60.00			2	60.00				
Stones					100	0.00						
Stream channels for mayfly test					20	2 631.60						
T-pieces for air tubing	10	4.90	10	4.90					20	9.80		
Test tube racks							12	1 188.00				
Test tube caps (16 mm)							250	85.00				
Test tubes, glass (16 × 150 mm)							250	625.00				
Thermometer	2	79.98	2	79.98	2	79.98	2	79.98	2	79.98	1	39.99
Trays (plastic)	2	172.80	2	172.80								
Vacuum filter apparatus					1	1 172.00			1	1 172.00	1	1 172.00
Vacuum pump (handheld)					1	750.00			1	750.00	1	750.00
Volumetric flask (100 mℓ)	2	45.96	2	45.96			2	45.96				
Volumetric flask (500 mℓ)	4	210.44	4	210.44								
Volumetric flask (1000 mℓ)							15	693.15	7	323.47	7	323.47
Wash bottle, plastic	2	32.00	2	32.00	2	32.00	3	48.00	2	32.00	2	32.00

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Water filter (dechlorinating)					1	597.00			1	597.00	1	597.00
Water pumps, submersible					20	850.00						
Weighing boats	2	0.89	2	0.89			2	0.89				
Total		**224 137.53**		**224 737.53**		**34 503.46**		**378 603.94**		**23 564.96**		**34 334.54**

Table A.2 Quantity and cost in Rands of equipment required to undertake the toxicological tests using the commercial test kits that were assessed in this report. Equipment listed here is that used by Toxsolutions Kits and Services. Software is listed as having no cost as open-source software is available that is suitable for the requirements of the tests. All costs are VAT exclusive.

	Algaltoxkit F		Daphtoxkit F magna		Daphtoxkit F pulex		Rotoxkit F short-chronic		Protoxkit F		Biotox	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Aeration stones			10	14.30	10	14.30	10	14.30				
Air pumps for fish tank			1	46.75	1	46.75	1	46.75				
Air tubing 5mm (25 m roll)			0.3	6.67	0.3	6.67	0.3	6.67				
Beakers, glass (50 mℓ)							6	60.00	6	60.00		
Beakers, glass (100 mℓ)	10	100.00										
Beakers, glass (250 mℓ)			4	102.00	4	102.00						
Centrifuge	1	5 000.00					1	5 000.00	1	5 000.00		
Centrifuge tubes, glass (50 mℓ)	10	1 694.00					10	1 694.00	10	1 694.00		
Chiller											1	25 800.00
Computer	1	6 000.00	1	6 000.00	1	6 000.00	1	6 000.00	1	6 000.00	1	6 000.00
Conductivity meter	1	3 329.00	1	3 329.00	1	3 329.00	1	3 329.00	1	3 329.00	1	3 329.00
Conductivity meter probe	1	738.00	1	738.00	1	738.00	1	738.00	1	738.00	1	738.00
Control valves for air tubing			10	10.50	10	10.50	10	10.50				
Cuvette rack											1	200.00

	Algaltoxkit F		Daphtoxkit F magna		Daphtoxkit F pulex		Rotoxkit F short-chronic		Protoxkit F		Biotox	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Deionising column for water	1	3 923.00	1	3 923.00	1	3 923.00	1	3 923.00	1	3 923.00	1	3 923.00
Dissolved oxygen meter and probe	1	5 394.00	1	5 394.00	1	5 394.00	1	5 394.00	1	5 394.00	1	5 394.00
Freezer (-18ºC)	1	22 970.00							1	22 970.00	1	22 970.00
Incubator			1	10 611.12	1	10 611.12	1	10 611.12	1	10 611.12		
Incubator, (lighted, simple, for Algaltoxkit)	1	5 000.00										
Light, counting, (for *Daphnia,* simple)			1	340.00	1	340.00						
Light, hatching			1	250.00	1	250.00	1	250.00				
Luminometer											1	34 500.00
Luminometer software and cabling											1	4 995.00
Magnifying glass			1	68.40	1	68.40						
Measuring cylinder (10 mℓ)	1	61.00										
Measuring cylinder (100 mℓ)			6	186.00	6	186.00					1	31.00
Measuring cylinder (250 mℓ)	1	59.00							1	59.00		
Measuring cylinder (500 mℓ)			1	78.25	1	78.25	1	78.25				

	Algaltoxkit F		Daphtoxkit F magna		Daphtoxkit F pulex		Rotoxkit F short-chronic		Protoxkit F		Biotox	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Micropipette (1-10 mℓ)			1	1 700.00	1	1 700.00	1	1 700.00	1	1 700.00		
Micropipette (100-1000 µℓ)	1	799.00	1	799.00	1	799.00	1	799.00	1	799.00	1	799.00
Microscope, dissecting (basic)							1	5 000.00				
Needles, dissecting			2	140.00	2	140.00	2	140.00	2	140.00		
pH meter	1	3 365.00	1	3 365.00	1	3 365.00	1	3 365.00	1	3 365.00	1	3 365.00
pH meter probe	1	633.00	1	633.00	1	633.00	1	633.00	1	633.00	1	633.00
Refrigerator (4ºC)	1	2 833.33	1	2 833.33	1	2 833.33	1	2 833.33	1	2 833.33	1	2 833.33
Software, spreadsheet	1	0.00	1	0.00	1	0.00	1	0.00	1	0.00	1	0.00
Software, statistical			1	0.00	1	0.00			1	0.00	1	0.00
Spectrophotometer (standard/10 cm cuvette)	1	30 000.00							1	30 000.00		
Stopwatch/timer											1	199.00
T-pieces for air tubing			10	4.90	10	4.90	10	4.90				
Thermometer	1	39.99	1	39.99	1	39.99	1	39.99	1	39.99	1	39.99
Volumetric flask (1000 mℓ)	1	46.21	1	46.21	1	46.21	1	46.21	1	46.21		
Vortex mixer (kits)							1	2 000.00				
Total		**91 984.53**		**40 659.42**		**40 659.42**		**53 717.02**		**99 334.65**		**115 749.32**

Table A.3 Quantity and cost in Rands of equipment required to maintain cultures of organisms used in the toxicological tests using indigenous organisms that are assessed in this report. Equipment listed here is that used in the laboratories of UCEWQ. All costs are VAT exclusive.

	***Daphnia pulex* culture**		***Caridina nilotica* culture**		**Algal culture**	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Aeration stones	10	14.30	39	55.77		
Air pumps for fish tank	1	46.75	26	1 215.50		
Air tubing 5mm (25 m roll)	0.4	8.89	2	44.46		
Aquarium tank (large)			13	1 658.80		
Aquarium tank (small)			13	1 885.00		
Autoclave					1	25 422.00
Balance, laboratory, coarse	1	2 109.00			1	2 109.00
Balance, laboratory, fine					1	11 086.50
Beakers, glass (600 mℓ)	3	78.00			5	130.00
Beakers, glass (3000 mℓ)	13	2 275.00				
Buckets (plastic, 25 ℓ)			2	63.08		
Bunsen burner					1	186.00
Conductivity meter			1	3 329.00		
Conductivity meter probe			1	738.00		

	Daphnia pulex culture		*Caridina nilotica* culture		Algal culture	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Control valves for air tubing	10	10.50	26	27.30		
Deionising column for water	1	3 923.00	1	3 923.00	1	3 923.00
Dissolved oxygen meter and probe			1	5 394.00		
Filter for aquarium (hang-on)			13	1 144.00		
Filter for aquarium (undergravel)			26	464.88		
Gas cylinder (LPG, 9 kg)					1	152.63
Graduated pipettes, glass (1 mℓ)					2	18.00
Graduated pipettes, glass (10 mℓ)					5	60.00
Graduated pipettes, glass (25 mℓ)	1	19.00			5	95.00
Haemocytometer					1	355.68
Heater, submersible, for aquarium (100 W)			26	1 272.70		
Heater/ magnetic stirrer	1	2 400.00			1	2 400.00
Incubator	1	10 611.12				
Incubator (lighted)					1	17 800.00
Light box (simple overhead light source)	1	200.00				
Magnifying glass	1	68.40				
Measuring cylinder (50 mℓ)					2	52.20

	Daphnia pulex culture		*Caridina nilotica* culture		Algal culture	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Measuring cylinder (1000 mℓ)	1	125.00			1	125.00
Micropipette (10-100 µℓ)					1	899.00
Micropipette (100-1000 µℓ)					1	799.00
Microplate reader					1	42 000.00
Microplate reader software					1	8 508.77
Microscope, compound					1	50 085.00
MilliQ water purification system	1	181 468.00			1	181 468.00
Needles, dissecting	5	350.00	5	350.00		
Net (fine nylon mesh)			26	143.00		
Paintbrush (fine-medium, soft)	2	7.02				
pH meter	1	3 365.00	1	3 365.00	1	3 365.00
pH meter probe	1	633.00	1	633.00	1	633.00
Photosynthetic photon flux density (PPFD) meter					1	3 580.00
Pipette bulb for pasteur pipette					5	45.00
Pipette bulb/pipette filler					2	178.00
Pipette canister					2	776.00

	Daphnia pulex culture		*Caridina nilotica* culture		Algal culture	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Pipettes, glass (10 mℓ)					10	120.00
Pipettes, glass (25 mℓ)					10	190.00
Reagent bottles, glass (100 mℓ)					2	50.00
Refrigerator (4°C)					1	2 833.33
Sample bottle, polyethylene (100 mℓ)					1	8.00
Schott bottles (100 mℓ)					10	156.00
Schott bottles (500 mℓ)					10	171.90
Schott bottles (1000 mℓ)					15	384.75
Silicone gun			1	13.50		
Spatula	2	280.00			2	280.00
Stirrer magnets	5	150.00			10	300.00
T-pieces for air tubing	10	4.90	26	12.74		
Test tube racks					10	990.00
Test/culture tube caps (16 mm)					250	85.00
Test/culture tubes, glass (20 × 150 mm)					250	1 230.00
Thermometer	1	39.99			4	159.96
Thermometer (aquarium)			26	142.48		

	Daphnia pulex culture		*Caridina nilotica* culture		Algal culture	
	No.	**Cost (R)**	**No.**	**Cost (R)**	**No.**	**Cost (R)**
Volumetric flask (1000 mℓ)					5	231.05
Wash bottle, plastic					3	48.00
Water filter (dechlorinating)			1	597.00		
Weighing boats	5	2.22			5	2.22
Total		**208 189.09**		**26 472.21**		**363 491.99**

Table A.4 Quantity and cost in Rands of materials required to undertake a toxicological test on one sample using the procedures utilizing indigenous organisms that are assessed in this report. Materials listed here are those used in the laboratories of UCEWQ. LPG gas is costed as the proportion of a full cylinder that is used. MilliQ water purifier cartridges/filters and water filter cartridge (dechlorinating) are costed in units of litres, with the cost being based on the number of litres that the filter can process before needing replacement. All costs are VAT exclusive.

	Daphnia pulex **lethality test**		***Daphnia pulex*** **reproduction test**		**Mayfly 10 day lethality test**		**Algal growth inhibition test**		***Caridina nilotica*** **juvenile lethality test**		***Caridina nilotica*** **10 day lethality test**	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Aluminium foil							0.5 roll	5.26				
Autoclave tape							0.5 m	0.71				
Boric acid							286 µg	<0.01				
Cadmium chloride	32.1 mg	0.01	790 mg	0.15			20 mg	<0.01				
Calcium chloride dihydrate							3.6 mg	<0.01				
Calcium sulphate dihydrate	30.2 mg	<0.01	609 mg	0.04								
Citric acid							600 µg	<0.01				
Cobalt (II) nitrate hexahydrate							4.94 µg	<0.01				
Copper sulphate (II) pentahydrate							7.9 µg	<0.01				
Cotton wool							0.5 roll	6.95				

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Detergent, laboratory (phosphate-free)	0.25 ℓ	1.82	0.25 ℓ	1.82	0.25 ℓ	1.82	0.25 ℓ	1.82	0.25 ℓ	1.82	0.25 ℓ	1.82
Dipotassium hydrogen phosphate							4 mg	<0.01				
DPD reagent pills	1	4.37	1	4.37	4	17.48	1	4.37	1	4.37	1	4.37
EDTA disodium salt dihydrate							100 µg	<0.01				
Ethanol (70%)					1 ℓ	6.31	0.5 ℓ	3.16				
Ferric ammonium citrate							600 µg	<0.01				
Filter paper	1	0.40	1	0.40	5	2.00			2	0.80	2	0.80
Hydrochloric acid (32%)	2.01 ℓ	44.11	2.10 ℓ	46.28			2.32 ℓ	51.05	2 ℓ	44.00	2 ℓ	44.00
Insulation tape							0.25 roll	0.32				
Lens tissue							5	2.25				
LPG gas refill (9 kg)							0.002	0.08				
Magnesium sulphate heptahydrate	61.8 mg	0.06	1.25 g	1.29			7.5 mg	0.01				
Manganese (II) chloride tetrahydrate							181 µg	<0.01				
Micropipette tips (100 µℓ)							96	16.22				
Micropipette tips (1000 µℓ)	60	15.48	60	15.48			60	15.48			100	25.80

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Microplates, sterile, polystyrene, 24 well							4	72.80				
Microscope slide coverslips							5	0.65				
Microscope slides							5	3.50				
MilliQ water purifier cartridges/filters	0.5 ℓ	0.31	1.5 ℓ	0.93			1 ℓ	0.62				
Parafilm (5.1 cm)	2 m	5.22	2 m	5.22			1 m	2.61	2 m	5.22	2 m	5.22
Pasteur pipettes, glass							10	2.92	1	0.29		
Petri dishes, sterile, polystyrene (90 mm)					10	7.60	10	7.60				
Pill vials (10 mℓ)					200	52.00						
Pill vials (20 mℓ)					100	29.00						
Pipette, plastic disposable (10 mℓ)					5	17.50						
Plastic sheet	1 m	0.18	1 m	0.18								
Potassium choride	2.01 mg	<0.01	40.6 mg	<0.01								
Sodium bicarbonate	48.3 mg	<0.01	975 mg	0.03			2 mg	<0.01				
Sodium hydroxide	0.8 g	0.09	16 g	1.79			40 g	4.48				

	Daphnia pulex lethality test		*Daphnia pulex* reproduction test		Mayfly 10 day lethality test		Algal growth inhibition test		*Caridina nilotica* juvenile lethality test		*Caridina nilotica* 10 day lethality test	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Sodium molybdate dihydrate							39 µg	<0.01				
Sodium nitrate							0.15 g	0.08				
Sodium thiosulphate	8 g	0.43	160 g	8.64			200 g	10.80				
Syringe filters (sterile, 0.22 µm, 13 mm dia)							5	15.00				
Syringes (25 mℓ, disposable)							2	2.40				
Tetramin flakes									1 g	0.84	5 g	4.19
Water filter cartridge (dechlorinating, carbon)					480 ℓ	39.00			5 ℓ	0.41	20 ℓ	1.63
Zinc sulphate heptahydrate							22.2 µg	0.00				
Total		**72.48**		**86.62**		**172.71**		**231.14**		**57.75**		**87.83**

Table A.5 Quantity and cost in Rands of materials required to undertake a toxicological test on one sample using the commercial test kits that are assessed in this report. Materials listed here are based on those used in the laboratories of Toxsolutions Kits and Services. All costs are VAT exclusive.

	Algaltoxkit F		Daphtoxkit F magna		Daphtoxkit F pulex		Rotoxkit F short-chronic		Protoxkit F		Biotox	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Algaltoxkit F kit	0.5	1 100.00										
Biotox kit											0.17	291.67
Cups, plastic, disposable (65 mℓ)	6	3.21					6	3.21	6	3.21	6	3.21
Cups, plastic, disposable (250 mℓ)	6	5.00	6	5.00	6	5.00						
Cuvettes, polystyrene, non-sterile											12	4.20
Daphtoxkit F magna kit			0.17	450.00								
Daphtoxkit F pulex kit					0.17	366.67						
Detergent, laboratory (phosphate-free)	0.1 ℓ	0.73	0.1 ℓ	0.73	0.1 ℓ	0.73	0.1 ℓ	0.73	0.1 ℓ	0.73	0.1 ℓ	0.73
DPD reagent pills	1	4.37	1	4.37	1	4.37	1	4.37	1	4.37	1	4.37
Gloves, latex, disposable	4	2.08	4	2.08	4	2.08	4	2.08	4	2.08	4	2.08
Micropipette tips (10 mℓ)			20	3.38	20	3.38	20	3.38	20	3.38		
Micropipette tips (1000 µℓ)	20	5.16	20	5.16	20	5.16	20	5.16	20	5.16	20	5.16
Nitric acid	0.2 ℓ	8.25	0.2 ℓ	8.25	0.2 ℓ	8.25	0.2 ℓ	8.25	0.2 ℓ	8.25	0.2 ℓ	8.25

	Algaltoxkit F		Daphtoxkit F magna		Daphtoxkit F pulex		Rotoxkit F short-chronic		Protoxkit F		Biotox	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Pipette, plastic disposable (10 mℓ)											20	70.00
Protoxkit F kit									0.33	650.00		
Rotoxkit F short-chronic kit							0.33	783.33				
Total		**1128.80**		**478.97**		**395.64**		**810.51**		**677.18**		**389.67**

Table A.6 Quantity and cost in Rands of materials to maintain cultures of organisms used in the toxicological tests using indigenous organisms that are assessed in this report for one month. Materials listed here are those used in the laboratories of UCEWQ. LPG gas is costed as the proportion of a full cylinder that is used. MilliQ water purifier cartridges/filters and water filter cartridge (dechlorinating) are costed in units of litres, with the cost being based on the number of litres that the filter can process before needing replacement. All costs are VAT exclusive.

	***Daphnia pulex* culture**		***Caridina nilotica* culture**		**Algal culture**	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Alfalfa food	4.35 tablets	0.80				
Aluminium foil					1 roll	10.52
Autoclave tape					2 m	2.84
Boric acid					572 µg	<0.01
Calcium chloride dihydrate					7.2 mg	0.01
Calcium sulphate dihydrate	7.83 g	0.52				
Citric acid					1.2 mg	<0.01
Cobalt (II) nitrate hexahydrate					9.88 µg	<0.01
Copper sulphate (II) pentahydrate					18.5 µg	<0.01
Cotton wool					0.5 roll	6.95
Detergent, laboratory (phosphate-free)	0.75 ℓ	5.46	0.75 ℓ	5.46	0.75 ℓ	5.46

	Daphnia pulex culture		*Caridina nilotica* culture		Algal culture	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Dipotassium hydrogen phosphate					8 mg	0.01
DPD reagent pill	10	43.70				
EDTA disodium salt dihydrate					200 µg	<0.01
Ethanol (70%)					1 ℓ	6.31
Ferric ammonium citrate					1.2 mg	<0.01
Gloves, latex, disposable					20	10.40
Gravel (for aquarium)			2.17 kg	7.36		
Hydrochloric acid (32%)	3.01 ℓ	66.15	3.01 ℓ	66.31	3.01 ℓ	66.15
Lens tissue					40	18.00
LPG gas refill (9 kg)					0.08	3.23
Magnesium sulphate					15 mg	<0.01
Magnesium sulphate heptahydrate	16.0 g	16.58				
Manganese (II) chloride tetrahydrate					362 µg	<0.01
Micropipette tips (100 µℓ)					20	3.38
Micropipette tips (1000 µℓ)					20	5.16
Microplates, sterile, polystyrene, 24 well					4	72.80

	Daphnia pulex culture		*Caridina nilotica* culture		Algal culture	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Microscope slide coverslips					40	5.20
Microscope slides					40	28.00
MilliQ water purifier cartridges/filters	120 ℓ	74.38			20 ℓ	12.40
Parafilm (5.1 cm)					1 m	2.61
Pasteur pipettes, glass					50	14.60
Petri dishes, sterile, polystyrene (90 mm)					50	38.00
Pipette, plastic disposable (10 mℓ)	10	35.00				
Potassium chloride	522 mg	0.03				
Silicone sealant (non-toxic)			0.5 tube	18.15		
Sodium bicarbonate	12.5 g	0.35			4 mg	<0.01
Sodium hydroxide	4 g	0.45	8 g	0.90	4 g	0.45
Sodium molybdate dihydrate					78 µg	<0.01
Sodium nitrate					0.3 g	0.16
Syringe filters (sterile, 0.22 µm, 13 mm dia)					5	15.00
Syringes (50 mℓ, disposable)					4	26.80

	***Daphnia pulex* culture**		***Caridina nilotica* culture**		**Algal culture**	
	Amount	**Cost (R)**	**Amount**	**Cost (R)**	**Amount**	**Cost (R)**
Tetramin flakes			200 g	167.50		
Trout pellets	54.8 g	4.81				
Water filter cartridge (dechlorinating, carbon)			650 ℓ	52.81		
Yeast	22.6 g	5.88				
Zinc sulphate heptahydrate					44.4 µg	<0.01
Total		**254.11**		**318.49**		**354.44**

Table A.7 Amount of time and labour costs in Rands for two skill ranks required to undertake a toxicological test on one sample using the procedures utilizing indigenous organisms that are assessed in this report. Time estimates presented here are derived from the practices of the laboratories of UCEWQ. All costs are VAT exclusive.

	Daphnia pulex **lethality test**		***Daphnia pulex*** **reproduction test**		**Mayfly 10 day lethality test**		**Algal growth inhibition test**		***Caridina nilotica*** **juvenile lethality test**		***Caridina nilotica*** **10 day lethality test**	
	Time	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**
Technical assistant	8.75 hr	325.06	47.25 hr	1 755.34	51.00 hr	1 894.65	4.08 hr	151.70	9.08 hr	337.45	11.58 hr	430.32
Technical officer	1.25 hr	54.54	1.25 hr	54.54	1.75 hr	76.35	1.75 hr	76.35	1.25 hr	54.54	2.75 hr	119.98
Total		**379.60**		**1809.88**		**1971.00**		**225.05**		**391.99**		**550.30**

Table A.8 Amount of time and labour costs in Rands for two skill ranks required to undertake a toxicological test on one sample using the commercial test kits that are assessed in this report. Time estimates presented here are derived from the practices of the laboratories of Toxsolutions Kits and Services. All costs are VAT exclusive.

	Algaltoxkit F		Daphtoxkit F magna		Daphtoxkit F pulex		Rotoxkit F short-chronic		Protoxkit F		Biotox	
	Time	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**
Technical assistant	1.92 hr	71.20	1.33 hr	49.53	1.33 hr	49.53	3.50 hr	130.03	1.50 hr	55.73	1.50 hr	55.73
Technical officer	1.08 hr	47.27	0.92 hr	39.99	0.92 hr	39.99	1.25 hr	54.54	1.25 hr	54.54	1.25 hr	54.54
Total		**118.47**		**89.52**		**89.52**		**184.57**		**110.27**		**110.27**

Table A.9 Amount of time and labour costs in Rands for two skill ranks required to maintain cultures of organisms used in the toxicological tests using indigenous organisms that are assessed in this report for one month. Time estimates presented here are derived based on the practices of the laboratories of UCEWQ. All costs are VAT exclusive.

	***Daphnia pulex* culture**		***Caridina nilotica* culture**		**Algal culture**	
	Time	**Cost (R)**	**Time**	**Cost (R)**	**Time**	**Cost (R)**
Technical assistant	35.42 hr	1 315.98	68.85 hr	2 557.65	24.28 hr	901.91
Technical officer	4.00 hr	174.52	4.00 hr	174.52	4.00 hr	174.52
Total		**1490.50**		**2732.17**		**1076.43**