supervising scientist report **182**

A review of the *eriss* Ecotoxicology Program

R van Dam

Australian Government
Department of the Environment and Heritage
Supervising Scientist

R van Dam – Environmental Research Institute of the Supervising Scientist, GPO Box 461, Darwin NT 0801, Australia

This report should be cited as follows:

van Dam R 2004. *A review of the **eriss** Ecotoxicology Program*. Supervising Scientist Report 182, Supervising Scientist, Darwin NT.

The Supervising Scientist is part of the Australian Government Department of the Environment and Heritage.

Supervising Scientist
Department of the Environment and Heritage
GPO Box 461, Darwin NT 0801 Australia

ISSN 1325-1554

ISBN 0 642 24389 1

Printed and bound in Darwin by uniprint NT

Contents

Executive summary vi

List of acronyms and abbreviations xi

Acknowledgments xii

Introduction 1

Background 1

Terms of reference 1

Approach 2

History 2

Previous reviews of ecotoxicology laboratory 3

1993 Review 3

1995 Review 4

Ranger ecotoxicology research 7

Uranium 7

Magnesium sulfate 12

Manganese 15

Ammonia 18

Pre-release toxicity testing 19

Linking ecotoxicological knowledge and biophysical pathways 21

Other ecotoxicology research 22

Alligator Rivers Region 22

Other research 23

External research funding 24

Research directions 25

Specific opportunities 33

Commercial ecotoxicology 36

Track record 36

The Northern Tropical/Sub-Tropical Region 37

The market in northern Australia 38

Prospective clients 40
Competition 42
Specific opportunities 43
Quality Assurance and Quality Control 44
Constraints 47
A proposed strategy 48

Laboratory issues 50
Metal contamination in toxicity tests 50
Flow-through water delivery system 52
Stock culturing 54
Refinement of test procedures 57
General laboratory procedures 59

Staffing 60
Existing resources 60
Implications 61
Requirements 62
Training and development 63

Concluding remarks 64

References 65

Appendices

Appendix A *eriss* Ecotoxicity Testing Discussion Paper (Bayliss et al 2003) 70
Appendix B Summary of uranium toxicity to local aquatic species in Magela Creek water 74
Appendix C Uranium toxicity PhD proposal – 2000 77
Appendix D Summary of 1992-93 magnesium toxicity studies (compiled by Rick van Dam, 1999) 79
Appendix E Manganese concentrations at selected sites around Ranger mine site 82
Appendix F Notes on a Northern Australian Ecotoxicology centre (prepared by Rick van Dam, January 2002) 85
Appendix G Example Sample Tracking / Chain-of-Custody form (from EVS Environment Consultants) 89

Appendix H Summary of investigations into sources of contamination and actions taken (compiled by Alicia Hogan, November 2003) 91

Appendix I Laboratory maintenance charts 93

Appendix J Recommendations for appointment of an SRS – Ecotoxicologist and contract extension for EA3 Laboratory Technician position 96

Appendix K Request for continuation of EA3 Laboratory Technician position 100

Tables

Table 1 Summary of chronic toxicity of uranium in Magela Creek water to local species 8

Table 2 Summary of toxicity of Mg^{2+} in Magela Creek water to local species 13

Table 3 Details of key funding bodies 26

Table 4 Developments and activities in the Northern Territory associated with the coastal and marine environment for which opportunities might exist for ecotoxicological research 31

Table 5 List of commercial projects undertaken by *eriss* ecotoxicology laboratory (1993–2003) 37

Table 6 Government departments/agencies/authorities relevant to industries or activities that may require ecotoxicology services 41

Table 7 Potential industry clients in the Northern Territory for the ecotoxicology laboratory 42

Table 8 Copper concentrations measured in different samples handled or treated in different ways 51

Table 9 Summary of implications of insufficient level of staffing in Ecotoxicology Program 61

Figures

Figure 1 Relationship of U toxicity to *Chlorella* sp. (as the IC50) to i. percent U bound to DOC (% U-DOC) and ii. percent uranyl ion 12

Figure 2 Map of Australia showing the major climate classifications 39

Figure 3 Map of Australia showing average surface sea temperatures 39

Executive summary

A review of the status and future strategic directions of the Environmental Research Institute of the Supervising Scientist (***eriss***) Ecotoxicology Program was undertaken between September 2003 and March 2004. The review encompassed all aspects of the uranium mining and non-uranium mining activities of the Ecotoxicology Program and considered both research and commercial opportunities. Specifically, the following terms of reference were addressed:

- Assess the status of and recommend directions for ***eriss*** ecotoxicological research; and
- Provide advice on and seek avenues for the commercial development of the existing ***eriss*** ecotoxicological laboratory and service.

In doing this, the review focused on the following areas:

- Previous reviews of the ecotoxicology laboratory
- Uranium mining ecotoxicology research
- Other ecotoxicology research
- Commercial ecotoxicology
- Existing laboratory issues
- Staffing

A total of 43 recommendations were made, as listed below.

Previous reviews of ecotoxicology laboratory

1. That the following (updated) recommendations from Baird (1996) be reconsidered:
   - That it is essential that an 'artificial water' control is included in all tests, and that this should be based on the water chemistry of Magela Creek during the wet season.
   - That retention pond water should be collected by composite depth sampling, and collections should be coordinated with Ranger mine scientists.
   - That staff from Ecotoxicology and Environmental Chemistry meet to discuss the procedures currently used in toxicity testing at ***eriss***, with regards to sample contamination issues.
   - Some measure of training in the statistics used in the toxicity tests should be given to those staff who have to interpret test data.

Ranger Ecotoxicology research

2. That the chronic toxicity of uranium to the duckweed, *L. aequinoctialis*, and the snail, *A. cumingi*, be assessed in the laboratory using the revised protocols (see Discussion on $MgSO_4$ and Recommendations 5 & 6) and combined with the existing chronic toxicity data to derive a revised uranium trigger value based on seven local species. *NB – some additional experimentation on the most appropriate culturing technique for the snail will be required.*
3. If, in the future, the development of and mining at Jabiluka proceeds, the toxicity of uranium in Ngarradj water should be assessed for at least three species previously used to assess uranium toxicity in Magela Creek water (preferably the three most sensitive species). The data should be compared with the toxicity of uranium in Magela Creek

water to the two species. If toxicity of uranium in Ngarradj water differs significantly from that in Magela Creek water, it is likely that further species would need to be tested and a Jabiluka site-specific trigger value derived as per the Ranger trigger value. Geochemical speciation modelling of uranium in Ngarradj and Magela Creek waters could be used as additional information/evidence in the decision making process.

4. That a study, possibly a postgraduate student project, be undertaken to fully assess and quantify the relationship between dissolved organic matter (DOM) and uranium bioavailability and toxicity in the context of Ranger mine and Magela Creek. The project should focus on the implications for risk assessment and link the results to dissolved organic carbon (DOC) concentrations in Magela Creek, including seasonal/temporal issues and consequences for retention pond discharges. Other relevant metals also could be assessed as part of the study.
5. That the toxicity of Mg (as $MgSO_4$) to the duckweed, *L. aequinoctialis*, be assessed in the laboratory using filtered, un-autoclaved natural Magela Creek water.
6. That the toxicity of Mg (as $MgSO_4$) to the snail, *A. cumingi*, be assessed in the laboratory using the revised protocol developed in 2002/03. *NB – some additional experimentation on the most appropriate culturing technique for the snails will be required.*
7. That two additional definitive tests are completed assessing the toxicity of Mg to the cladoceran, *M. macleayi*.
8. That the data for the above-mentioned species are combined with the existing site-specific toxicity data for Mg to derive a new Mg trigger value (in the absence of a Ca amelioration effect).
9. That the relative contributions of the Mg^{2+} cation and $SO4^{2-}$ anion to $MgSO_4$ toxicity be determined for two additional species, most likely the duckweed, *L. aequinoctialis*, and the snail, *A. cumingi*.
10. That the influence of Mg:Ca ratio on the toxicity of Mg to the duckweed, *L. aequinoctialis*, and the snail, *A. cumingi*, being amongst the more sensitive species tested, be assessed in the laboratory;
11. That these data and those already existing for hydra are used to determine a 'trigger' Mg:Ca ratio below which adverse effects on aquatic biota would not be expected;
12. That the protective ability of the trigger Mg:Ca ratio is then confirmed for the remaining species in a series of smaller experiments; and
13. That the concentration at which Mg eventually becomes toxic at the trigger Mg:Ca ratio be quantified for all six species, and the data used to derive a Mg trigger value (99% protection) at the trigger Mg:Ca ratio.
14. That the toxicity of Mn to local species be assessed using Magela Creek water (preferably at < pH 6) as diluent in range-finding experiments that span a broad concentration range (eg 30, 300, 3000 and 30 000 μg Mn/L). In the absence of a fish chronic toxicity test it is recommended that toxicity be assessed using the green alga, *Chlorella* sp., the cladoceran, *C. dubia*, and the green hydra, *H. viridissima*, the measured responses of each of which represent chronic toxicity endpoints.
15. That the toxicity of the RO permeate be assessed once the full-scale treatment plant is fully operational, regardless of whether the composition of the permeate is similar to that from the pilot plant.

16. That there be an assessment or review (most likely desk-top) of the likely impact that changes in pH and temperature of water in RP1 and MBL bund would have on the percentage of unionised ammonia and therefore ammonia toxicity.

17. That relevant ***eriss*** and Energy Resources Australia (ERA) staff discuss and document a formal agreement for the conduct of the annual pre-release toxicity testing program. The agreement should clearly outline the pre-release toxicity testing process including the context and objectives of the testing program; the responsibilities of each party; timing of and notification for the commencement of testing in relation to anticipated water discharges; toxicity testing details including test species/endpoints, dilution water and statistical treatment of data; reporting requirements and timelines including internal publication; procedures for notification of problems relating to the testing program; and any other special conditions.

Research directions

18. That ecotoxicology research directions over the next 12 to 18 months focus on:
    - Ranger (and Jabiluka) related research;
    - Strategically focused improvement and development of test methods and laboratory procedures; and
    - Future strategic research planning efforts.
19. That future ecotoxicology research directions (ie beyond 18 months) focus on:
    - Ecological risk assessment development;
    - Mining/point source impacts research across northern Australia;
    - Developing a marine ecotoxicology capability (~5 year timeframe); and
    - Provision of training, both nationally and internationally.
20. That linkages and collaborations with other institutions are considered in the context of the possible establishment of a Northern Australian or National Ecotoxicology Consortium/Centre, and that the concept of the consortium to be identified within the strategic review as a significant opportunity for ***eriss***.

Specific opportunities

21. That the fluroxypyr and metsulfuron methyl risk assessment proposal be revised as suggested in the report text and submitted for an *ARC – Linkage* or *ARC – Discovery* Grant in 2004.

22. That efforts to resubmit the ***eriss***/UTS/NSW EPA/SKM salinity ecotoxicology/risk assessment proposal are kept active.

23. That contact be maintained with the National Oceans Office (NOO), primarily Dr Ilse Kiessling, in order to maximise the potential for future opportunities.

24. That discussions continue towards a collaborative research proposal for developing an appropriate framework or model for assessing risks and impacts of mine water discharges on freshwater ecosystems in tropical Australia, with a view to submitting for funding in 2004. The next step needs to be a group workshop to discuss priority issues and agree on a strategic research theme.

25. That the prospect of developing a research project proposal based on endocrine disrupting compounds (EDCs) in tropical aquatic ecosystems be further investigated, with discussions continued with Dr Mika Peck, Dr Louis Tremblay and Associate Professor Richard Lim.

26. That a funding proposal be completed by mid-2005 for the development (or first stage of development) of appropriate methodologies for assessing contaminant risks and impacts to tropical marine species.

Commercial Ecotoxicology

27. That a check-list be developed, listing all essential major tasks for commercial projects, from proposal through to reporting and close-out stage.

28. That efforts be undertaken with ***eriss***/SSD to develop the capacity to accurately cost commercial projects and monitor and review project costs.

29. That a reference toxicity testing program be implemented for all routinely used species. This should probably be implemented in a tiered manner, with an aim to have established reference toxicant Control Charts for each species within 2 years.

30. That formal sample tracking procedures be developed and implemented for commercial projects.

31. That a short but formal marketing and business development plan be produced based on the information in this section, which details relevant activities, timelines and monitoring/evaluation procedures.

Laboratory issues

32. That the frequency and magnitude of Cu, Zn and Al contamination in test samples and the concentrations of these metals in Darwin tap water (DTW) and filtered Darwin tap water (FDTW) is closely monitored and that if necessary further discussions are held with relevant experts and/or further investigations are undertaken to try to further reduce the contamination incidence.

33. That further consideration and investigation is given to the health problems experienced by *M. macleayi* cultures in FDTW.

34. That, as part of the ecotoxicology laboratory's quality system, a procedure be implemented to ensure that all mathematical calculations required for culturing and toxicity testing purposes are double-checked by another member of the laboratory staff.

35. That further discussions with relevant staff and external experts are held to consider and agree on approaches for ensuring a consistent supply of fish eggs/larvae when needed, for toxicity testing purposes.

36. That the *Chlorella* sp. culture be treated for removal of bacterial contamination.

37. That the feasibility of reducing the per replicate test volume for the *Chlorella* sp. population growth test from 50 ml in a 250 ml Erlenmeyer flask to 15 ml (in a glass scintillation vial) be investigated.

38. That the 2003–04 Ecological Risk Assessment training budget include an allocation for the ecotoxicology laboratory staff to undergo the appropriate image analysis software training by Leica staff.

39. That the minimum NO_3 and PO_4 concentrations required to be added to Magela Creek water (MCW) in order to consistently exceed the minimum acceptability criterion for *L. aequinoctialis* control growth over 96 h be determined.

40. That differences in reproduction and associated reproductive variability between similarly sized snails (*A. cumingi*) of known similar age and similarly sized snails of unknown age need to be determined, in order to establish the minimum culturing requirements/effort for the *A. cumingi* test.

41. That the existing *M. mogurnda* toxicity test be adapted for using a rainbowfish species, probably black-striped rainbowfish (*Melanotaenia nigrans*) or chequered rainbowfish (*Melanotaenia splendida inornata*). This could be completed as a student (eg Honours) project.

Staffing

42. That the Ecotoxicology research program staffing allocation be maintained, at a minimum, at two laboratory-based personnel and a senior research ecotoxicologist.

43. That further training of the Ecotoxicology research program staff be sought in several key areas, notably toxicity testing protocols, experimental design and statistics, ecological risk assessment and aspects of commercial toxicity testing, as relevant to their level and duties.

The key outcomes and conclusions of the review included the following:

- There is still substantial uranium mining ecotoxicology research required to fill key information gaps, and this research will probably take a minimum of 18 months to 2 years to complete.
- The ecotoxicological resource and associated body of knowledge that has developed at ***eriss*** over the past 20 years represents a key asset for the Supervising Scientist, and one for which every effort should be made to retain and utilise well beyond the completion of the uranium mining ecotoxicology research.
- Beyond the completion of the uranium mining research, ecotoxicology activities need to be a mix of both externally funded research and commercial projects. If possible, the primary function of the ecotoxicology laboratory should remain that of research, with a secondary but nonetheless significant focus on securing commercial activities. For this model to be effective will require the establishment of productive, long-term relationships and partnerships with key research institutions, government agencies and industry groups.
- The ecotoxicology laboratory appears geographically, competitively and logistically (ie infrastructure, equipment, procedures) well-placed to secure and successfully undertake more commercial work. However, communication with key consulting firms, State and Territory government departments and industries needs to be initiated and maintained in order to maximise opportunities.

As a final note, it is promising to note that by the completion and publication of this review, seven of its recommendations had already been addressed (ie Recommendations 5, 6, 7, 8, 17, 39 and 42) while a further 19 were being addressed or had been agreed to address (ie Recommendations 2, 3, 9, 10, 11, 13, 14, 15, 18, 23, 24, 25, 27, 28, 32, 33, 38, 40 and 43).

List of acronyms and abbreviations

ANSTO	Australian Nuclear Science and Technology Organisation
ANZECC	Australian and New Zealand Environment and Conservation Council
ARC	Australian Research Council
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
ARR	Alligator Rivers Region
ARRTC	Alligator Rivers Region Technical Committee
CDU	Charles Darwin University (formerly Northern Territory University NTU)
EDC	endocrine disrupting compound
ERA	Energy Resources Australia
eriss	Environmental Research Institute of the Supervising Scientist
DBIRD	Department of Business, Industry and Resource Development (Northern Territory)
DIPE	Department of Infrastructure, Planning and Environment (Northern Territory)
DjB	Djalkmara Billabong
DOM	dissolved organic matter
DTW	Darwin tap water
FDTW	filtered Darwin tap water
LOEC	Lowest-Observed-Effect Concentration
MCW	Magela Creek water
MLAA	Magela Land Application Area
NATA	National Association of Testing Authorities
NCTWR	National Centre for Tropical Wetland Research
NOEC	No-Observed-Effect Concentration
NOO	National Oceans Office
NSW EPA	New South Wales Environment Protection Authority
OSS	Office of the Supervising Scientist
RP1	Ranger Uranium Mine Retention Pond 1
SSD	Supervising Scientist Division
UTS	University of Technology Sydney

Acknowledgments

The author would like to thank Dr Arthur Johnston, Dr Max Finlayson and Dr Peter Bayliss of the Supervising Scientist Division (SSD) for supporting the need for and implementing this review. Other SSD staff that provided important information, opinion, comment and advice included Dr Andreas Bollhöfer, Caroline Camilleri, Alicia Hogan, Dr Chris Humphrey, Shelly Iles, Carolyn Lord, Dr Paul Martin, Joan Mount, Tida Nou, Dr Claudia Sauerland and Alex Zapantis. Former PhD students at SSD, Clint McCullough and Dr Mika Peck, also supplied data and information. Additionally, information, opinion, comment and advice was sought from outside SSD, specifically, from Professor Donald Baird (Environment Canada, University of New Brunswick, Canada), Dr Carlos Barata (Consejo Superior de Investigaciones Científicas; CSIC, Spain), Dr Ross Hyne (New South Wales Environment Protection Authority, Sydney), Michael Lawton (Northern Territory Department of Infrastructure, Planning and Environment, Darwin), Dr Scott Markich (Australian Nuclear Science and Technology Organisation, Sydney), John Milsom (Energy Resources of Australia, Jabiru), Associate Professor David Parry (Charles Darwin University, Darwin), Dr Greg Rippon (Department of the Environment and Heritage, Canberra) and Pamela Sanders (Northern Territory Department of Business, Industry and Resource Development). In addition, advice on specific aspects within the review and comments on the final report by Professor Doug Holdway (University of Ontario Institute of Technology, Canada; and one of the seven independent members of ARRTC) was greatly appreciated. Finally, Ann Webb (SSD) is thanked for her meticulous editorial work.

A review of the *eriss* Ecotoxicology Program

R van Dam

Introduction

Background

The Alligator Rivers Region Technical Committee (ARRTC) is an independent scientific advisory panel with the primary aim of ensuring that the quality of the science used in the research into, and assessment of, the protection of the environment from the impact of uranium mining in the Alligator Rivers Region, Northern Territory, Australia, is of an appropriately high standard. At its 10th meeting (September 2002), ARRTC requested information on the future of the ecotoxicology laboratory and associated resource implications for the Environmental Research Institute of the Supervising Scientist (***eriss***) (ARRTC 2002). Although ***eriss*** has committed to maintaining the ecotoxicology laboratory for pre-release toxicity testing of retention pond waters (ARRTC 2002), other ecotoxicological research on uranium (U) and magnesium (Mg) in relation to Ranger uranium mine (Ranger) is thought to be in its latter stages. Essentially, ARRTC asked:

'*so what next…*' and '*…what are the resource implications?*'

In an initial response to this request, ***eriss*** produced a Discussion Paper (Bayliss et al 2003; see Appendix A) that outlined very briefly the current status of, and discussed 3 future options for, the ecotoxicology laboratory: *Closure*; *Maintain status quo*; and *Maintain status quo and expand commercially*. The third option (*Maintain status quo and expand commercially*) was recommended, and associated opportunities, constraints and recommendations were identified and briefly discussed. At its 11th meeting (February 2003) ARRTC responded by noting that a small amount of ecotoxicological research was still occurring and that ecotoxicological issues should be addressed through the strategic planning process, and then considered as part of the overall priority list of actions (ARRTC 2003). There was agreement that the future of the ecotoxicology laboratory was an operational matter for the Supervising Scientist (P Bayliss, ***eriss***, pers comm).

Thus, a comprehensive review and assessment of the existing status of Ranger-related ecotoxicology research and the future strategic direction of the ecotoxicology laboratory was initiated and undertaken between September 2003 and March 2004.

Terms of reference

The initial terms of reference (TOR) for the project, which was intended to take three months, were as follows:

1. Assess the status of and recommend directions for ***eriss*** ecotoxicological research;
2. Provide advice on and seek avenues for the commercial development of the existing ***eriss*** ecotoxicological laboratory and service;

3. Represent ***eriss*** as required and support research planning activities connected with the above tasks; and if time permitted
4. Assist in the development of ***eriss*** ecological risk assessment and conceptual models.

Following further discussions it was agreed that the focus of the review be placed on TOR 1 and 2 while incorporating relevant aspects of TOR 3.

Approach

In order to effectively address the terms of reference for the review (see following section), the following tasks and activities were undertaken:

- Review of the recommendations and outcomes of previous reviews of the ecotoxicology laboratory;
- Review of the extent of historical ecotoxicology research at ***eriss***, both that related to and not related to Ranger or uranium mining in general;
- Discussions with relevant Supervising Scientist Division (SSD) staff on:
  - the historical ecotoxicology research and subsequent requirements for additional Ranger-related ecotoxicology research;
  - options for the future strategic direction, both research and commercial, of the ecotoxicology laboratory; and
  - Specific issues of importance to the ongoing functioning of the ecotoxicology laboratory, primarily aspects relating to staffing resources and laboratory procedures;

  and
- Discussions with key stakeholders, particularly in the Northern Territory, to gauge the type and extent of future research and commercial opportunities for the ecotoxicology laboratory.

This review has attempted to acknowledge and expand upon all the issues identified by Bayliss et al (2003). It is anticipated that the findings and recommendations of this review, which are presented throughout the report and also listed in full in the Executive Summary, will be used to develop a strategic plan for the future of the ecotoxicology laboratory.

History

The history of the ***eriss*** ecotoxicology laboratory has been documented in detail elsewhere (see Johnston 1994, Hyne et al 1996, ANZECC & ARMCANZ 2000, Riethmuller et al 2003), A brief chronological description of key stages and issues only is provided here.

A formal research program investigating the toxicity of mine contaminants to aquatic fauna in the Alligator Rivers Region was established in 1982. The program initially focused on the toxicity of single metals such as copper, zinc and lead, and laid the foundation for the development of an extensive whole effluent toxicity (WET) testing program, designed to monitor the toxicity of pre-release wastewaters from Ranger.

In 1986, a specific program was initiated to develop a suite of standard protocols for assessing the aquatic toxicity of pre-release wastewaters (ie for WET testing). The program assessed the suitability of approximately nineteen local aquatic species, with eight species being found to have potential as test organisms. These were used to develop nine initial protocols.

In 1991 the number of protocols in use was reduced from nine to four and they were subsequently documented in full by Hyne et al (1996) and Markich and Camilleri (1997).

From 1991 to 1994 the four protocols and their use by the ecotoxicology laboratory were registered by the National Association of Testing Authorities (NATA).

From the mid 1990s to 2000, the Ranger Environmental Laboratory undertook pre-release toxicity testing of their retention pond waters following appropriate test protocol handover, staff training and under informal supervision by ***eriss*** ecotoxicology staff.

By 1995/96, following the broadening of ***eriss***'s functions under the *Environment Protection (Alligator Rivers Region) Act 1978* in 1994, the ecotoxicology laboratory research focus broadened to include the assessment of non-mining related issues (eg herbicide ecotoxicity) and the application of ecological risk assessment approaches.

In 2001, following the implementation of the Supervising Scientist routine Ranger monitoring program and rationalisation of the Ranger Environmental Laboratory's functions, the responsibility of the pre-release toxicity testing of Ranger retention pond waters was transferred back to the ***eriss*** ecotoxicology laboratory from the Ranger Environmental Laboratory.

In 2001–2002 new ecotoxicology laboratories were constructed as part of the new ***eriss*** facility in Darwin, with relocation of staff and testing facilities occurring in mid 2002.

The strategic changes since 1995/96 and the adoption of risk-based approaches for uranium mining-related toxicity assessments (in line with the *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*; ANZECC & ARMCANZ (2000)) stimulated the development of further toxicity testing protocols, namely for aquatic plants. Thus, there currently exists a suite of six routine toxicity tests. The full suite of toxicity testing protocols was recently fully documented by Riethmuller et al 2003.

Whilst the laboratory was re-established in Darwin in mid-2002 it had been without a senior ecotoxicologist since September 2001, although a less senior research position for an ecotoxicologist was vacant at the time.

Previous reviews of ecotoxicology laboratory

There have been two previous formal reviews of the ecotoxicology laboratory and/or associated laboratory activities. These were carried out in 1993 and 1995, by Mr Vince Brown (Brown 1994) and Dr Donald Baird (Baird 1996), respectively. This section summarises the key findings of the reviews and examines the extent to which the recommendations were adopted.

1993 review

Brown (1994) focused on the adequacy of the procedures used in the ecotoxicology laboratory, the relevance and applicability of NATA requirements and a number of research issues relating to the influence of feeding in cladocerans on metal toxicity.

Overall, Brown (1994) provided substantial personal opinion and informal suggestions, but few formal recommendations. Key outcomes/recommendations of the review are listed below. Where relevant, a brief description of the extent to which the outcome/recommendation was adopted follows (in bold italics).

1. The documentation and practical application of the pre-release toxicity testing procedures for *Hydra viridissima*, *Moinodaphnia macleayi* and *Mogurnda mogurnda* were appropriate and of high quality.

2. Given the necessity for strict Quality Assurance in an ecotoxicology laboratory, it was recommended that access to laboratory equipment and facilities be restricted to nominated and authorised persons only.

   The need for Quality Assurance and an 'authorised persons only' laboratory was recognised and attempts were made with varied success over the years. The current ecotoxicology laboratory in Darwin was designed such that there is no flow-through of general traffic or need for unauthorised persons to enter without appropriate reason and approval.

3. It was of some concern that the number of species used for pre-release toxicity testing had been reduced to three species and that this might compromise the ability to protect the downstream environment from retention pond water discharges.

   While, the number of routine toxicity testing protocols used at eriss has increased since the review, the number of species/tests used for pre-release toxicity testing has not changed. Given (i) the integrated approach to protecting the downstream aquatic environment from retention pond water discharges (ie a combined approach of chemical specific guidelines, pre-release toxicity testing, in-stream toxicity monitoring and long-term fish and macroinvertebrate community structure monitoring), and (ii) the fact that the three species used for pre-release toxicity testing represent different taxonomic groups and trophic levels, the program as it stood was seen as acceptable.

4. While the laboratory organisation and procedures necessary to meet NATA requirements were laid out thoughtfully and in considerable detail, NATA registration was not considered necessary or particularly relevant to the site-specific and complex nature of the ecotoxicological work performed at the time by the laboratory.

   NATA registration for the ecotoxicology laboratory and pre-release toxicity testing protocols was not maintained after 1994, although this was the result of the loss of technical and senior staff at this time rather than a deliberate choice.

5. An earlier proposal by Dr Donald Baird and ***eriss*** staff to develop a rapid toxicity test based on a response such as feeding inhibition in a cladoceran was considered inappropriate in the context of ensuring the protection of the aquatic environment downstream of Ranger, namely because such a test would represent an acute response when in fact longer-term (chronic) responses were the major issue.

   In contrast to Brown's opinions, the development of a rapid-response test using M. macleayi was later considered a potentially worthwhile investment for eriss's ecotoxicology program and research investigating its feasibility and application was undertaken with varied success between 1998 and 2000 (see Orchard 2000, Smith 2001, Orchard et al 2002).

1995 review

Baird (1996) undertook a consultancy that addressed a number of TOR related to the status and development of the Wetland Protection and Management program, including the following task related to the ecotoxicology laboratory:

Advise on the development of the ***eriss*** ecotoxicity laboratory by reviewing current day-to-day procedures, testing protocols and their adaptability for broader testing purposes, develop a program for publishing past research and assess future toxicological research directions and the development of further tests linked to environmental monitoring needs.

Clear recommendations were made and are provided as dot points below. Again, where relevant, a brief description of the extent to which the recommendation was adopted follows (in bold italics).

- That the Toxicology section should be given a separate area for culture of invertebrates and the performance of tests, and that this should include a constant temperature facility with power backup.

  See response to second outcome/recommendation from Brown (1994) review.

- That the cladoceran culture system be changed from an individual culture system to a bulk culture system, which is simpler, and produces healthier animals.

  Attempts at establishing and maintaining laboratory bulk culture systems revealed that M. macleayi performed better and could be better tracked when in individual cultures. While individual animal cultures have been maintained, back-up bulk cultures are also kept.

- That the diet of the cladocerans be investigated, to see if a less complex, algal diet could be used.

  Research on suitable food sources for M. macleayi cultures undertaken in the early 1990s (see Hyne 1991, Rippon & le Gras 1993), before Baird's review, indicated that this species is largely a bacterial feeder. This also has been found to be the case for other small cladoceran species (eg Ceriodaphnia dubia; Mount & Norberg 1984, as cited by Hyne 1991). From my experience, M. macleayi performs best in terms of survival and/or reproduction when fed on a mixed diet consisting of a fermented food (bacterial) suspension (ie FFV) and an algal species (Chlorella sp.), than on only one of these food sources. In the aquatic environment, M. macleayi is likely to feed on both bacteria and algae (and probably other sources of organics carbon). Thus, it is appropriate that both types of food are provided for culturing and toxicity testing. Thus, except for its interest as a research question, it is not considered essential that this recommendation be adopted.

- That it is essential that an 'artificial water' control is included in all tests, and that this should be based on the water chemistry of Magela Creek during the wet season.

  This was not implemented. Although an artificial Magela Creek water was developed (by ANSTO) and was suitable for larval fish and hydra tests (cladocerans did not perform well in the artificial water) it has not been routinely used as a standard control for toxicity tests. This could be reconsidered, probably in conjunction with the implementation of a regular reference toxicity testing program (see Commercial Ecotoxicology, below).

- That tests should be re-engineered to reduce their duration without compromising their sensitivity.

  The hydra 6 day population growth test was reduced to 4 days (96 h) after comparison of statistical results after 6 and 4 days indicated no change in test sensitivity.

- That there is a need for a rapid response test.

  See response to fifth outcome/recommendation from Brown (1994) review. It should be noted that although this test was fully developed and documented (see Orchard 2000, Smith 2001, Orchard et al 2002), its potential inconsistency limited its application for pre-release toxicity testing and it has not been a routinely used test.

- That RP2 water should be collected by composite depth sampling, and collections should be coordinated with Ranger mine scientists.

  According to ecotoxicology laboratory staff, this recommendation has not been adopted. This appears to be more due to the fact that staff from the mining company had been handed the responsibility of pre-release toxicity testing by the time the recommendation was made. With eriss again responsible for this work, this issue could be reconsidered for future collections of retention pond water to be used for toxicity testing.

- That an in-house workshop involving staff from Toxicology and Chemistry be organised to evaluate the procedures currently used in toxicity testing at ***eriss***.

  This workshop was not held. However, given recent sample contamination issues in the ecotoxicology laboratory, it may be considered worthwhile for Ecotoxicology and Environmental Chemistry staff to meet and discuss general laboratory procedures, sources of contamination and procedures for minimising contamination.

- That the question of non-monotonic responses be addressed, and that effluent concentrations are routinely checked.

  To my knowledge, the issue of how to treat non-monotonic responses was not formally addressed, except that such decisions were and are based on professional judgement, and depended on the nature and objective of the testing. Currently, the statistical software used by the ecotoxicology laboratory, ToxCalc, notifies the user of non-monotonic responses and requires a decision on whether the test should proceed as a one-tailed or two-tailed test.

- Some measure of training in the statistics used in the toxicity tests should be given to those staff who have to interpret test data.

  Only one staff member from the ecotoxicology laboratory in 1995 is still at eriss, and this person did receive formal and informal (ie on-the-job) training in statistics. Currently, the ecotoxicology laboratory staff have an understanding of statistics equal or greater to that required for an Ecotoxicology Honours project. In addition, further training and development in statistics is being actively sought (see Staffing, below).

- That the draft technical memorandum on the development of the toxicity test procedures (Hyne et al 1992) be published without further revision.

  This report was published as SSR110 (Hyne et al 1996).

- That the work on test animal husbandry be collated and written up as an internal report to avoid information loss.

  This information has been collated and is included as Appendices in the recently published toxicity testing protocols document SSR173 (Riethmuller et al 2003).

- That the study on the impact of KEMMAT spraying of *Salvinia* on floodplain billabongs be written up for peer-reviewed scientific publication.

  This research was not written up for peer-review publication, but is available as an Internal Report (IR159; Finlayson et al 1994).

Baird (1996) also suggested that the future development of the ecotoxicology research program focus on risk assessment including, in relation to Ranger mine impacts, an ability for ***eriss*** to assess the bioavailability and toxicity of uranium in floodplain sediments (also see Baird et al 1995). From 1996, a formal risk assessment component was introduced to the ecotoxicology research program which now forms the basis of the program, while a sediment toxicity test was developed and used to assess the toxicity of uranium in sediments (see Peck 2000, Peck et al 2002) although has not been in routine use since (see *Ranger Ecotoxicology Research*, below).

In general, the recommendations from previous reviews have been adopted or defensibly argued, resulting in ongoing improvements to the day-to-day functioning and research capabilities of the ecotoxicology laboratory. However, several recommendations from Baird (1996) remain unaddressed and should be reconsidered.

Recommendation

1. That the following (updated) recommendations from Baird (1996) be reconsidered:
   - That it is essential that an 'artificial water' control is included in all tests, and that this should be based on the water chemistry of Magela Creek during the wet season
   - That retention pond water should be collected by composite depth sampling, and collections should be coordinated with Ranger mine scientists.
   - That staff from Ecotoxicology and Environmental Chemistry meet to discuss the procedures currently used in toxicity testing at ***eriss***, with regards to sample contamination issues.
   - Some measure of training in the statistics used in the toxicity tests should be given to those staff who have to interpret test data.

Ranger ecotoxicology research

As its heading indicates, this section reviews the status of ecotoxicological research undertaken in relation to the Ranger uranium mine. However, it is not the intention to fully document and describe the full accumulated body of work since the early 1980s, but rather to focus on key research on the major contaminants of concern that enables the Supervising Scientist to ensure the protection of the aquatic environment of Magela Creek downstream of the mining operations. In addition, the ecotoxicological research that has been undertaken in relation to other mining operations in the ARR, specifically Nabarlek but also Rockhole Mine and Coronation Hill, is acknowledged but not considered as a part of this review.

Uranium

Historically, the major aquatic contaminant of concern in Ranger retention pond waters was considered to be uranium. Thus, with the exception of the development of site-specific

toxicity testing procedures (for an overview see Riethmuller et al 2003), the majority of Ranger-related ecotoxicology research has focused on this toxicant. Since the mid 1980s, approximately 21 freshwater species local to the Alligator Rivers Region (ARR) have been assessed for uranium toxicity (two plant, two cnidarian, one mussel, six crustacean and 10 fish species) (van Dam 2002). It should be noted that although the toxicity of uranium to local species has been extensively studied (as described below) not all of the studies have had direct relevance to the discharge of Ranger retention pond waters. Objectives of the research have varied, and included the assessment of acute or chronic uranium toxicity in a site-specific context, determination of the influence of specific physico-chemical parameters (eg alkalinity, water hardness) on uranium toxicity and assessment of inter-population differences in the sensitivity of aquatic species to uranium. Nevertheless, the over-arching emphasis on this topic of research has been to determine concentrations of uranium in Magela Creek downstream of Ranger that would not result in adverse effects on aquatic biota (ie water quality guidelines or trigger values).

Ranger site-specific assessment of uranium toxicity

In the context of the protection of the ecologically important aquatic ecosystems downstream of Ranger, it is those studies that have assessed uranium toxicity to local species in local Magela Creek water that are of greatest value. These studies are summarised in Appendix B. Unfortunately, much of the data relate to *acute* effects of uranium, which, given the importance of the region are of limited applicability. From the existing data, it is evident that acute toxic effects of uranium to various aquatic species were not usually observed at concentrations below 1000 μg/L (see Appendix B). Of greater relevance are sub-lethal responses to prolonged exposure to lower concentrations of uranium (ie chronic effects) as a result of retention pond water discharges. Of all the organisms that have been assessed, chronic toxicity data exist for only five species. Recently, the no-observed-effect concentration (NOEC) data for these species (table 1) were used to determine a site-specific water quality guideline trigger value for Magela Creek downstream of Ranger using the risk-based statistical extrapolation method developed for the ANZECC and ARMCANZ (2000) Water Quality Guidelines. With the Magela Creek catchment considered such an ecologically important area, a high protection trigger value was derived that would protect at least 99% of species as per the approach of the ANZECC and ARMCANZ (2000) Water Quality Guidelines. Thus, the resultant trigger value for uranium of 6 μg/L (Hogan et al 2003) has been adopted as a water quality 'limit' for downstream of Ranger (van Dam et al 2002). This approach is considered a major advance on the previous method for determining a 'safe' concentration or addition of uranium in Magela Creek, where an (arbitrary) assessment factor was simply applied to the lowest ecotoxicity value.

Table 1 Summary of chronic toxicity of uranium in Magela Creek water to local species

Species	Test endpoint	NOEC (μg L^{-1})	Reference
Chlorella sp.	Cell division rate (72 h)	117[a]	Hogan et al (2003)
Moinodaphnia macleayi	Reproduction (3 brood)	18 [a]	***eriss*** unpubl, Semaan et al (2001)
Hydra viridissima	Population growth (96 h)	183 [a]	ARRRI (1988), Hyne et al (1992)
Mogurnda mogurnda	Mortality (7 d exposure / 7 d post-exposure)	400	Holdway (1992)
Melanotaenia splendida inornata	Mortality (7 d)	810	Holdway (1992)

[a] Toxicity values represent geometric means from ≥ 2 tests

Although the data for uranium meet the minimum requirements as specified in the ANZECC and ARMCANZ (2000) Water Quality Guidelines, there are major limitations associated with using only five data points for such an approach. For example, the uncertainty surrounding the value is very large (ie 95% confidence limits: 0.3 – 103 μg/L) while the value also displays a marked degree of model dependency (see van Dam 2002, Hogan et al 2003). In fact, BurrliOZ, the Water Quality Guidelines software used to calculate trigger values, warns of the inherent uncertainties when a dataset of fewer than eight values is selected to derive a trigger value. Ideally, assessment of the chronic toxicity of uranium to several additional species would probably increase confidence in the site-specific trigger value for uranium. This was the subject of a successful Australian Postgraduate Award PhD proposal in the ecotoxicology program in 2000 (see Appendix C), but the prospective student candidate did not accept the scholarship. Further justifying the need for additional species to be assessed, two of the five species used in the existing derivation are fish, which were substantially less sensitive to uranium than the other species tested (see Appendix B), which, given the small sample size, may be having a disproportionate influence on the final value.

Further developments in site-specific toxicity test protocols in the ecotoxicology laboratory since the above-mentioned PhD proposal mean that assessment of two additional species, the duckweed, *Lemna aequinoctialis*, and the snail, *Amerianna cumingi*, could be undertaken with minimal additional developmental work. The inclusion of these species would also add two additional taxonomic groups to the list and would provide added rigour as well as stakeholder (including the broader community) confidence in the trigger value.

Recommendation

2. That the chronic toxicity of uranium to the duckweed, *L. aequinoctialis*, and the snail, *A. cumingi*, be assessed in the laboratory using the revised protocols (*see Discussion on $MgSO_4$ and Recommendations 5 & 6*) and combined with the existing chronic toxicity data to derive a revised uranium trigger value based on seven local species. *NB – some additional experimentation on the most appropriate culturing technique for the snail will be required*.

Jabiluka site-specific assessment of uranium toxicity

Were the Jabiluka mine to proceed in the future, there would be some ecotoxicological issues associated with the potential for controlled or uncontrolled discharge of retention pond waters into Ngarradj (Swift Creek) and, more specifically, the toxicity of uranium in Ngarradj waters, which are characteristically around pH 5 and could result in substantially different toxicity to that observed in the *circa* pH 6 waters of Magela Creek (see summary below in *Other uranium toxicity research* of effects of pH on uranium toxicity). Given SSD's commitment to ensure the completion of all research required to ensure the protection of the surrounding environment if Jabiluka was to proceed, the toxicity of uranium in Ngarradj water to local freshwater species needs to be assessed. As a minimum, there is a need to confirm whether the uranium toxicity data for Ranger (ie using Magela Creek water) are appropriate for setting trigger values or limits for Jabiluka. This could be achieved by assessing and comparing the toxicity of uranium in Ngarradj water and Magela Creek water to perhaps two of the species previously used to derive the uranium trigger value for Ranger (eg the two most sensitive species – *M. macleayi*, *Chlorella* sp.). The results could be used to determine the suitability of existing data or the need to more comprehensively assess the toxicity of uranium in Ngarradj water.

Recommendation

3. If, in the future, the development of and mining at Jabiluka proceeds, the toxicity of uranium in Ngarradj water should be assessed for at least three species previously used to assess uranium toxicity in Magela Creek water (preferably the three most sensitive species). The data should be compared with the toxicity of uranium in Magela Creek water to the two species. If toxicity of uranium in Ngarradj water differs significantly to that in Magela Creek water, it is likely that further species would need to be tested and a Jabiluka site-specific trigger value derived as per the Ranger trigger value. Geochemical speciation modelling of uranium in Ngarradj and Magela Creek waters could be used as additional information/evidence in the decision making process.

Sediment toxicity test

From 1997 to 2000, a PhD student developed a lethal and sub-lethal sediment toxicity test using a local sediment-dwelling chironomid, *Chironomus crassiforceps* (Peck 2000, Peck et al 2002). The test was used to assess the toxicity of uranium in acid-sulfate sediments characteristic of the Magela Creek floodplain, which acts as the major depositional zone of the Magela Creek catchment. In initial water-only experiments, the toxicity of uranium to *C. crassiforceps* was determined to be relatively low, with 72-h LC_{50}s at pH 4 and pH 6 being 58 mg/L and 36 mg/L, respectively (Peck et al 2002). In whole-sediment toxicity tests, uranium was found to have no sub-lethal effect on *C. crassiforceps* at all levels expected in the environment (ie up to 5000 mg U/kg dry sediment). Given that chironomids are generally considered among the more sensitive sediment toxicity testing species, these results provide good evidence that sediment-bound uranium does not represent a high risk to sediment-dwelling organisms in the acid-sulfate sediments of the Magela floodplain. Thus, it would not seem necessary to undertake further research in this area.

Other uranium toxicity research

Some research on the effect of various environmental parameters on uranium toxicity has been undertaken, primarily to gain a greater understanding of the major factors affecting uranium bioavailability and toxicity and to assist in the development of broadly applicable tropical freshwater quality guidelines. In particular, between 1996 and 2000, two ANZECC funded projects (an Honours and a Masters project collaboration between ***eriss*** and the Australian Nuclear Science and Technology Organisation (ANSTO)) assessed the influence of key physico-chemical parameters on uranium toxicity to tropical freshwater species (Franklin et al 1998, Riethmuller et al 2000). Additional research in this area has also been undertaken by the Environment Division of ANSTO (Markich 1998, Charles et al 2002).

pH

Hyne et al (1992) assessed the influence of pH on the toxicity of uranium to *H. viridissima*. No toxicity was observed at pH 8.5 (at a uranium concentration of 1000 µg/L), while adverse effects on survival and population growth were observed between pH 5 and 7. Markich et al (1996), measuring valve movement responses of the tropical freshwater mussel, *Velesunio angasi*, observed that the sub-lethal toxicity of uranium was 5-fold greater at pH 5 than at pH 6, and concluded that the differences in toxicity were due to changes in uranium speciation. In contrast, Franklin et al (1998) found that the toxicity of uranium to the green alga, *Chlorella* sp. was lower by almost 2-fold at pH 5.7 compared to pH 6.5, with increased competition at the lower pH between uranium and H^+ for binding sites being postulated as the mechanism. Similarly, experiments using the chironomid, *C. crassiforceps*, found that acute

uranium toxicity was markedly lower at pH 4 than at pH 6 (see *Sediment toxicity test*, above). Although uranium bioavailability and toxicity is obviously affected by surface water pH, it is also clear that the exact relationship has not been well characterised and is likely to be different for different species.

Water hardness

Riethmuller et al (2000) assessed the influence of true water hardness and alkalinity on the toxicity of uranium and copper to green hydra (*H. viridissima*) and purple-spotted gudgeon (*M. mogurnda*). It was hoped that the project would lead to the development of quantitative relationships and algorithms to account for water hardness effects, however, results were variable. A 50-fold increase in water hardness resulted in a 2-fold decrease in the toxicity of uranium to *H. viridissima*, but had no effect on the toxicity of uranium to *M. mogurnda*. In a more recent study, Charles et al (2002) found that a 50-fold increase in water hardness resulted in a 5-fold decrease in the toxicity of uranium to *Chlorella* sp. Overall, the evidence suggests that increasing water hardness does reduce uranium toxicity and that this is primarily due to competition between uranium, Ca and/or Mg for binding sites rather than changes in speciation.

Alkalinity

Two studies have assessed the influence of alkalinity (independent of pH and hardness) on uranium toxicity to local aquatic organisms (Markich et al 1996, Riethmuller et al 2000). Toxicity of uranium to *V. angasi* at pH 5 was inversely proportional to alkalinity, with a 5-fold increase in alkalinity reducing toxicity by 20% (Markich et al 1996). In contrast, a 25-fold increase in alkalinity did not influence the toxicity of uranium to *H. viridissima* at pH 6. The conflicting results could be attributed to inter-species differences or the different pH levels (Riethmuller et al 2000). From the available data, little can be concluded about the influence of alkalinity on the toxicity of uranium to local aquatic species.

Dissolved organic matter

The need to understand the influence of dissolved organic mattter (DOM) on uranium toxicity has previously been raised (by Dr Arthur Johnston and Dr Barry Noller, formerly of the NT Department of Mines & Energy, now Deputy Director of the National Research Centre for Environmental Toxicology) as a crucial information gap. Although this has not been addressed comprehensively, a recent study on the toxicity of uranium to the green alga *Chlorella* sp. in local Magela Creek water (Hogan et al 2003) has gone some way to assessing this. Variability in toxicity test results for uranium in Magela Creek water was largely explained by differences in the percentage of uranium bound to dissolved organic carbon (DOC) as predicted by geochemical speciation modelling (HARPHRQ) (ie there was a strong negative relationship between % U-DOC and uranium toxicity; $r^2 = 0.988$, n = 4, $P = 0.012$, figure 1). Not surprisingly, the increase in U-DOC was associated with a decrease in the percentage of the free uranyl ion (UO_2^{2+}; $r^2 = 0.977$, n = 4, $P = 0.023$, figure 1). Hogan et al (2003) also showed that a four-fold reduction in the toxicity to the alga of uranium in natural Magela Creek water compared to synthetic Magela Creek water (ie simulating the inorganic composition of Magela Creek water but containing no organic component) was associated with a four-fold reduction in the percentage of UO_2^{2+}. Supporting these results, Markich et al (1996) observed that the toxicity of uranium to *V. angasi* was substantially ameliorated in synthetic Magela Creek water with increasing concentration of DOC, in the form of a synthetic fulvic acid. These results indicate that DOM in Magela Creek water (or its synthetic equivalent) is a major determinant of the bioavailability and toxicity of uranium to aquatic biota, yet it remains the least studied physico-chemical variable to date. Therefore, it would be highly desirable to undertake a comprehensive study, probably as a postgraduate student

project, to fully understand and quantify the influence of DOM on uranium (and other metals) bioavailability and toxicity to a range of freshwater species.

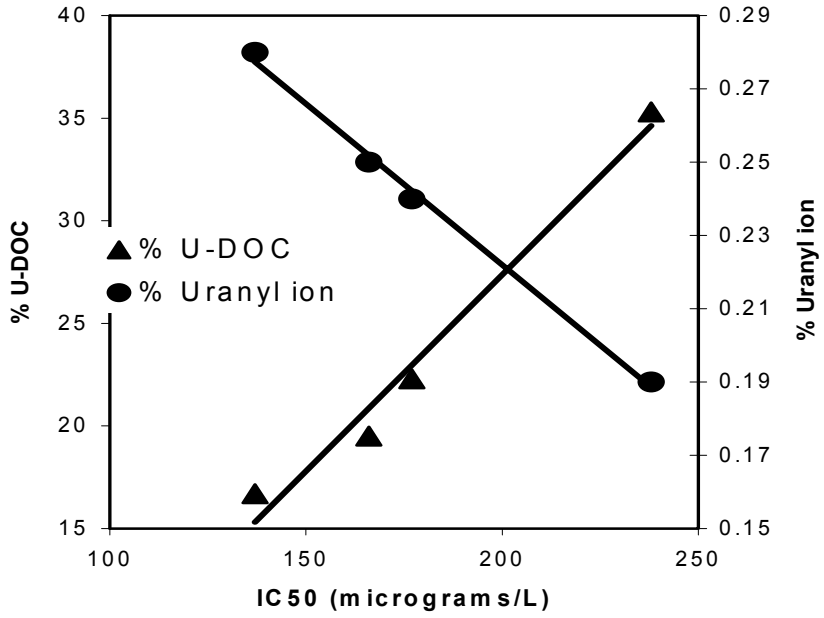

Figure 1 Relationship of U toxicity to *Chlorella* sp. (as the IC50) to i. percent U bound to DOC (% U-DOC) and ii. percent uranyl ion (from Hogan et al 2003)

The above types of studies provide understanding of the specifics of uranium speciation, bioavailability and toxicity rather than the means for specific protective measures (cf. site-specific toxicity testing and pre-release toxicity testing). Thus, in the context of the need to ensure the protection of the aquatic ecosystems downstream of Ranger, they are not of direct relevance. Subsequently, with the exception of DOM, there probably is no need for ***eriss*** to undertake any further research on the influence of physico-chemical parameters on uranium toxicity.

Recommendation

4. That a study, possibly a postgraduate student project, be undertaken to fully assess and quantify the relationship between DOM and uranium bioavailability and toxicity in the context of Ranger mine and Magela Creek. The project should focus on the implications for risk assessment and link the results to DOC concentrations in Magela Creek, including seasonal/temporal issues and consequences for retention pond discharges. Other relevant metals also could be assessed as part of the study.

Magnesium sulfate

Limited laboratory-based research on the toxicity of $MgSO_4$ was undertaken in the early 1990s. A summary of the work was compiled in 1999 and is provided in Appendix D. Of note were the following recommendations:

> The research … is insufficient to derive a high confidence trigger value for Mg or for a Mg:Ca ratio. Further research would need to be undertaken on more species and also assessing the toxicity

of different Mg concentrations at different Mg:Ca ratios, or at least at Mg:Ca ratios characteristic of Magela Ck water at 009.

…

Further research should be undertaken to assess the toxicity of Mg at a range of Mg:Ca ratios that are relevant to Magela Ck water at 009. In addition, worst case scenarios could also be assessed. Such assessments should be carried out for at least three aquatic species.

In 1999, a PhD was commenced to more comprehensively assess the toxicity and risks of $MgSO_4$ to aquatic biota local to Magela Creek. The project, which is in its final stages, has been complemented by additional laboratory toxicity testing efforts by ***eriss*** ecotoxicology laboratory staff. In the laboratory, the toxicity of $MgSO_4$ to six local species was assessed, although several of these test series are incomplete (see below). Additional tests using *H. viridissima* only, confirmed that the Mg^{2+} cation (not the SO_4^{2-} anion) was the primary cause of toxicity, although this should be confirmed for several other species. The toxicity of Mg to local aquatic species is summarised in table 2.

There existed some uncertainty about initial Mg toxicity results for *L. aequinoctialis*, because the natural Magela Creek water used for the test diluent was autoclaved prior to test commencement. This may have degraded the natural organic components of the water and hence modified the bioavailability and toxicity of Mg. This aspect was discussed with staff and efforts were made to assess the ability to run the tests in un-autoclaved creek water and the associated effect this has on Mg toxicity (hence, no need for a formal recommendation). Results from the experiments using un-autoclaved creek water demonstrated that the Lemna test is viable, and thus, the toxicity of Mg was reassessed and is presented in table 2.

Table 2 Summary of toxicity of Mg^{2+} in Magela Creek water to local species

Species	Test endpoint	Toxicity (mg L^{-1}) [a]		Status of results
		LOEC	NOEC	
Chlorella sp.	72-h Cell division rate	143	84	Final
Lemna aequinoctialis	96-h Population growth	3.1	1.8	Final
Amerianna cumingi	96-h Reproduction	2.3[c]	2.0[d]	Interim [b]
Moinodaphnia macleayi	3-brood Reproduction	18	10	Interim [b]
Hydra viridissima	96-h Population growth	4.6	2.2	Final
Mogurnda mogurnda	96-h Mortality	LC_{50} = 41 (NOEC equivalent = 8.3) [e]		Final

[a] Toxicity values represent geometric means of results from ≥2 tests, except for *M. macleayi*, which represent results from one test.

[b] See text for discussion

[c] Value represents IC_{20} value

[d] Value represents IC_{10} value

[e] NOEC equivalent obtained by applying a correction factor of 5 (for essential metals) to the LC_{50} value (ANZECC & ARMCANZ 2000)

There also exists some uncertainty about the toxicity of Mg to the snail, *A. cumingi*. Due to the snail laboratory test having to be adpated from creekside monitoring protocols, the resultant experimental design was quite coarse. For example, large test volumes restricted the test design to only two replicates per treatment, whilst test organisms were sourced from cultures in large outdoor tubs where conditions were uncontrolled. Consequently, the results were highly variable with very little statistical power and failed to provide a robust assessment of Mg toxicity. However, the protocol was refined in 2002–03 with substantial success. Controlled laboratory culturing, the ability to isolate and use animals from individual cohorts as test animals, a reduction in test volume to allow the addition of a third replicate and

testing in a more controlled laboratory environment greatly reduced the variability in the number of embryos produced, this being the test endpoint (***eriss***, unpublished data; also see *Laboratory Issues*, below). Although this work is yet to be written-up, examination of the raw data confirm that the snail test appears to have been suitably adapted to more controlled laboratory conditions, and greatly improved in the process. Therefore, it is appropriate that the toxicity of Mg to *A. cumingi* be reassessed using the revised protocol. Prior to this commencing, there may need to be some additional experiments investigating/quantifying the cost-benefits of culturing snails *en masse* in large outdoor tubs (ie as done for creekside monitoring tests) versus controlled laboratory culturing of single cohorts of snails.

Due to on-going problems with *M. macleayi* cultures since the ***eriss*** relocation to Darwin (See *Laboratory Issues*, below), only one of three required valid definitive tests has been completed. Investigations were underway to determine the cause(s) of the culturing problems (see *Laboratory Issues*, below), and, once resolved, two additional definitive tests assessing the toxicity of Mg will need to be completed.

The final laboratory toxicity data will be used to determine a water quality guideline trigger value (99% protection) for Mg. However, because the laboratory experiments did not account for the potential ameliorative effect of calcium (Ca – which is also found in Ranger retention pond waters at elevated levels) on Mg toxicity, the trigger value is not directly relevant to the Ranger situation. To address this, two laboratory experiments were undertaken to determine the influence of Ca concentration on Mg toxicity to *H. viridissima* with the aim of determining a Mg:Ca ratio below which adverse effects would not be observed. Results demonstrated that below a Mg:Ca ratio of approximately 10, a Mg concentration of 10 mg/L did not exhibit significant toxicity. While this has been sufficiently characterised for hydra, it would be desirable if the same could be completed for additional species, but noting that **full** assessments of all the other five species are probably not required. In this instance, it would be most appropriate to assess an additional two of the more sensitive species and determine a 'trigger' Mg:Ca ratio below which adverse effects on aquatic biota would not be expected based on assessment of two species. The protective ability of the trigger Mg:Ca ratio could then be confirmed for the remaining species in a series of smaller experiments. In addition, it may be necessary to quantify the concentration at which Mg eventually becomes toxic at the trigger Mg:Ca ratio. It is likely that such an effect would be an osmotic response rather than a toxic response. Nevetheless, if the toxicity of Mg at the trigger Mg:Ca ratio were assessed for all six species, the resultant data could be used to derive a trigger value for Mg when the Mg:Ca ratio is at or below the trigger ratio.

Recommendations

5. That the toxicity of Mg (as $MgSO_4$) to the duckweed, *L. aequinoctialis*, be assessed in the laboratory using filtered, un-autoclaved natural Magela Creek water.
6. That the toxicity of Mg (as $MgSO_4$) to the snail, *A. cumingi*, be assessed in the laboratory using the revised protocol developed in 2002/03. *NB – some additional experimentation on the most appropriate culturing technique for the snails will be required.*
7. That two additional definitive tests are completed assessing the toxicity of Mg to the cladoceran, *M. macleayi*.
8. That the data for the above-mentioned species are combined with the existing site-specific toxicity data for Mg to derive a new Mg trigger value (in the absence of a Ca amelioration effect).

9. That the relative contributions of the Mg^{2+} cation and SO_4^{2-} anion to $MgSO_4$ toxicity be determined for two additional species, most likely the duckweed, *L. aequinoctialis*, and the snail, *A. cumingi*.
10. That the influence of Mg:Ca ratio on the toxicity of Mg to the duckweed, *L. aequinoctialis*, and the snail, *A. cumingi*, being amongst the more sensitive species tested, be assessed in the laboratory;
11. That these data and those already existing for hydra are used to determine a 'trigger' Mg:Ca ratio below which adverse effects on aquatic biota would not be expected;
12. That the protective ability of the trigger Mg:Ca ratio is then confirmed for the remaining species in a series of smaller experiments; and
13. That the concentration at which Mg eventually becomes toxic at the trigger Mg:Ca ratio be quantified for all six species, and the data used to derive a Mg trigger value (99% protection) at the trigger Mg:Ca ratio.

Manganese

Background

Since mid-2001, a steep rise in Mn concentrations has been observed in at least one bore (MC20) in the Magela Land Application Area (MLAA). Groundwater Mn concentrations in Bore MC20 of 20 000 to 50 000 μg/L have been regularly recorded during this period and has coincided with a decrease in groundwater pH range from around 4.5–5.5 to 3.9–4.5 (see Appendix E, Figure D-1). Energy Resources of Australia (ERA), in its 2001/2002 Annual Environmental Management Report (ERA 2002) and at a specific meeting held with OSS staff on 11 February 2003 to discuss the issue, stated that this acidification was probably due to the oxidation of reduced forms of S, Fe and Mn following a lowering of the water table associated with a reduction in the water application rates. Regardless of the direct cause, these Mn concentrations represent some cause for concern given that MC20 is situated within the dominant flow path of water (in a north-easterly direction) towards Magela Creek.

Further concern has been expressed at the concentrations of Mn in Corridor Creek surface waters (A Johnston, SSD, pers comm), with concentrations up to 800 μg/L being recorded in early 2003 (see Appendix E, figure D-2). In addition, a Mn 'spike' of around 1200–1300 μg/L was recorded in Coonjimba Billabong (which receives wet season overflow water from RP1) in December 2002, persisting for at least a month before declining to below 200 μg/L by late January 2003 (see Appendix E, figure D-3). As with groundwater at Bore MC20, the increase in Mn concentration in Coonjimba Billabong coincided with a decrease in pH from around pH 7 to pH 4. The above event corresponded to the onset of flows in Magela Creek and, thus, was probably a result of the oxidation of sulfides and reduced forms of Fe and Mn following the first flows into Coonjimba Billabong. Although essentially a natural process, the magnitude of the Mn spike appears to be unprecedented in the 20-plus year monitoring period of Coonjimba Billabong. Further, the peak Mn concentration exceeded the ANZECC and ARMCANZ (2000) 99% protection trigger value for Mn of 1200 μg/L.

It is important to note that there does not yet appear to have been a concomitant increase in Mn concentrations in Magela Creek downstream (ie at 009) of the sites/sampling points discussed above, particularly Bore MC20. It has been suggested that the groundwater Mn may be precipitating in association with iron in a number of permanent groundwater-derived pools

that occur between the location of Bore MC20 and the creek channel (M Iles, ***oss***, pers comm). The pools represent a surface expression of the groundwater table, which has risen substantially due to Ranger's long-term irrigation practices. The fate of the elevated groundwater Mn may be elucidated by an anticipated project to investigate the vertical and lateral migration of radionuclides and metals/trace elements within the MLAA (P Martin, ***eriss***, pers comm).

Historically, spikes in Mn concentrations in Magela Creek occur at the beginning of the wet season and usually again at the end of the wet season, reflecting periods when groundwater surface expression is greater. While these spikes have been characteristically higher downstream of the minesite than upstream (see Appendix E, Figure D-4), based on data since 1991, the Ranger limit at 009 of 32 μg/L has been exceeded less than 1% of the time (see Appendix E, Figure D-5). Further, it should be noted that the Mn limit for Ranger is around 2 orders of magnitude more conservative (ie more protective) than the ANZECC and ARMCANZ (2000) 99% protection trigger value for Mn of 1200 μg/L.

The Minutes of the above-mentioned 11 February meeting, state that '***eriss*** has indicated that the derivation of a local guideline (limit) value for Mn will be undertaken by a program of ecotoxicological testwork scheduled for the 2003/04 financial year.' However, the necessity or otherwise of this should first be gauged by a literature review of the aquatic toxicity of Mn and factors affecting Mn toxicity. Outcomes of such a review should drive decisions about the type and extent of site-specific Mn toxicity assesment that needs to be undertaken. Therefore, a brief summary of the aquatic toxicity of Mn is provided below.

Aquatic toxicity of Mn

Mn is a common and essential biological metal, the toxicity of which is considered low compared to other trace metals. It is more soluble and hence more mobile under low pH and reducing conditions. Acute Mn toxicity (ie as EC/LC_{50}s) to species representing 6 taxonomic groups (macrophyte, crustacea, insecta, mollusca, amphibia, fish) has been reported at concentrations between 3680 μg/L to 4 540 000 μg/L (Stubblefield et al 1997, ANZECC & ARMCANZ 2000, Markich et al 2000). Chronic Mn toxicity (expressed as IC_{25} or NOEC values) to species representing 3 taxonomic groups (alga, crustacea, fish) has been reported at concentrations between 1270 μg/L to 9990 μg/L, over the pH range 6.75–8.4 (Stubblefield et al 1997, ANZECC & ARMCANZ 2000, Lasier et al 2000). Based on these data, the more sensitive species to Mn were the fathead minnow, *Pimephales promelas*, and the cladoceran, *Daphnia magna*, although this may be related to the long exposure period to which these species were subjected (ie 672 h for both species – as reported by ANZECC & ARMCANZ 2000).

A number of studies have assessed the influence of physico-chemical factors on Mn toxicity. Stubblefield et al (1997) found that Mn toxicity (as the IC_{25}) to brown trout decreased about 2-fold with a 15-fold increase in water hardness. A similar relationship between water hardness and Mn toxicity has also been reported for the freshwater amphipod, *Hyalella azteca*, and the cladoceran *Ceriodaphnia dubia*, although water hardness was not kept independent of alkalinity (Lasier et al 2000). Markich et al (2000) reported no change in Mn toxicity to *V. angasi* over a pH range of 5–6. However, other studies have reported conflicting results on the influence of pH on Mn toxicity to aquatic organisms, making it difficult to generalize about the relationship. Nevertheless, Mn is known to be soluble and mobile at low pH. Markich et al (2000) also reported no change in Mn toxicity over a model fulvic acid (employed to simulate the metal binding capacity of natural DOM) concentration range of 0–7.91 mg/L. Similar results were reported by Rouleau et al (1994) for brown trout in the

presence (at 5 mg/L) or absence of humic acid, suggesting that natural DOM plays a small role in the bioavailability and toxicity of Mn.

Scant data exist on the toxicity of Mn to local species. Markich et al (2000) assessed the effect of Mn on valve movement responses of the mussel, *V. angasi*, reporting a 48 h EC_{50}, minimum detectable effect concentration (MDEC; a LOEC analogue) and 10% bounded effect concentration (BEC_{10}; a NOEC analogue) of 29.6, 18.1 and 17.1 mg/L, respectively. In addition, the acute toxicity of Mn in Magela Creek or Ja Ja Billabong water to several fish species (Marjorie's hardyhead, carp gudgeon, black-striped rainbowfish and chequered rainbowfish) has also been assessed with 96 h LC_{50} values ranging from 10 200 μg/L to >500 000 μg/L (as cited in Markich & Camilleri 1997). Given the ultra-low water hardness (median Mg and Ca concentrations of 0.64 mg/L and 0.52 mg/L, respectively; Klessa 2000) and relatively low pH (*circa.* 25% of measured pH values fall below pH 6; Klessa 2000) of Magela Creek water, characteristics that could potentially result in higher Mn toxicity than has previously been reported, further site-specific assessment would seem necessary.

Site-specific assessment of Mn toxicity

Summarising from the above information, the need for site-specific assessment of Mn toxicity needs to be considered in the context of the following issues:

1 Evidence from the literature suggests the acute and chronic toxicity of Mn to aquatic biota is low – lower than concentrations previously recorded downstream of Ranger (at 009);

2 The current limit for Mn downstream of Ranger of 32 μg/L is approximately two orders of magnitude more conservative than the ANZECC & ARMCANZ (2000) 99% protection trigger value of 1200 μg/L, and since 1991 has been exceeded less than only 1% of the time;

3 Very high Mn concentrations (ie >20 000 μg/L) in groundwater that flows through the MLAA within the dominant flow path of water towards Magela Creek could represent a hazard to local aquatic biota of Magela Creek;

4 The Mn 'spike' of over 1300 μg/L in Coonjimba Billabong during December 2002/January 2003 exceeded the ANZECC & ARMCANZ (2000) 99% protection trigger value and is in the order of concentrations reported to cause chronic toxicity to at least two species of aquatic organisms, and therefore may be of toxicological concern; and

5 Too few toxicity data exist for local species in local Magela Creek water to be able to predict with high confidence that no adverse effects would be expected.

The first two issues provide some confidence that Mn downstream of Ranger is not of toxicological concern. However, the last three issues create some uncertainty around such a conclusion and, thus, provide sufficient basis on which to recommend some form of site-specific ecotoxicological assessment. This should focus on chronic toxicity and, in the first instance need only be concerned with determining whether Mn concentrations in the vicinity of those reported above are toxic to local aquatic biota in Magela Creek water. If toxicity were to be observed, a full scale Mn toxicity assessment program to derive a site-specific trigger value for Mn downstream of Ranger might be considered necessary.

Recommendation

14. That the toxicity of Mn to local species be assessed using Magela Creek water (preferably at < pH 6) as diluent in range-finding experiments that span a broad concentration range (eg 30, 300, 3000 and 30 000 μg Mn/L). In the absence of a fish chronic toxicity test it is recommended that toxicity be assessed using the green alga, *Chlorella* sp., the cladoceran, *C. dubia*, and the green hydra, *H. viridissima*, the measured responses of each of which represent chronic toxicity endpoints.

Ammonia

Background

Ammonia has emerged as a potential contaminant of concern following a proposal by ERA to treat Ranger process water to a standard suitable for discharge to the receiving environment. Water treatment, which involves lime treatment, carbonation, microfiltration/reverse osmosis (RO) and biopolishing (ie wetland filtration), produces a final permeate stream that is essentially a dilute solution of ammonium sulfate. Following a pilot plant trial in 2001, ammonia concentrations in the final permeate were estimated to be between 10–20 mg/L (as total ammonia). This concentration of ammonia is too high for direct release to receiving catchments on the Ranger lease so the permeate will first be passed through constructed wetlands designed to maximize the amount and rate of ammonia removal (through nitrification of ammonia to nitrate, and denitrification of nitrate to nitrogen gas). Wetlands for 'biopolishing' of the permeate have been constructed in the RP1 and Corridor Creek catchments, the outflows of which will enter RP1 and the Corridor Creek bunds (Brockman bund, MBL bund and Sleepy Cod bund), respectively, before being discharged (with various levels of control) in the wet season to Coonjimba and Georgetown Billabongs, respectively, and into Magela Creek. Hence, the need to understand the toxicity of the final permeate, and specifically, ammonia to local aquatic species.

Toxicity of treated waste water from pilot plant

In December 2001 a sample of the pilot process water treatment plant was assessed for toxicity using the *M. macleayi* reproduction, *M. mogurnda* survival and *H. viridissima* population growth tests (Camilleri et al 2002). The permeate sample contained 18 mg/L ammonia. The only other compounds present in potentially toxic concentrations (as assessed against ANZECC & ARMCANZ (2000) trigger values) were copper at 5.8 μg/L and zinc at 5.4 μg/L. The LOEC and NOEC (from the most sensitive of the three species; *M. macleayi* and *H. viridissima*) for the permeate were 32% and 10%, respectively. The investigators, assuming the majority, if not all, of the toxicity in the permeate would have been due to ammonia, derived a NOEC for ammonia of 1.7 mg/L, that being the ammonia concentration in the 10% permeate treatment. This NOEC value has been proposed as a limit (ie maximum permissible concentration) for ammonia at the Performance Monitoring Locations (RP1 weir and MBL bund), although an ***oss*** Discussion Paper on ERA's draft application to discharge RO permeate noted that 'In interpreting the results of the ecotoxicological testing of the whole effluent, concentrations of individual constituents in the NOEC water cannot be used directly to set a trigger value for ammonia. Rather, the results are used to specify the minimum dilution required for the effluent'. Although decisions such as these are yet to be finalised as ERA is yet to submit a formal application to discharge the RO permeate, the ***oss*** position is appropriate (for example, uranium trigger values are not derived from results of retention

pond water whole effluent toxicity testing; they are derived from uranium-specific toxicity test data). The ***oss*** Discussion Paper recommends that '…Toxicology tests should be carried out on the final plant output to establish that the composition of the effluent is similar to that used previously in the toxicity testing'. Again, this is appropriate, although it is essential that toxicity of the RO permeate again be assessed once the full-scale treatment plant is fully operational, regardless of whether the composition of the permeate is similar to that from the pilot plant. In addition, there should be some form of assessment or review that discusses the likely impact that changes in pH and temperature of water in RP1 and MBL bund would have on the percentage of unionised ammonia and therefore, ammonia toxicity.

Recommendations

15. That the toxicity of the RO permeate be assessed once the full-scale treatment plant is fully operational, regardless of whether the composition of the permeate is similar to that from the pilot plant.
16. That there be an assessment or review (most likely desk-top) of the likely impact that changes in pH and temperature of water in RP1 and MBL bund would have on the percentage of unionised ammonia and therefore ammonia toxicity.

Pre-release toxicity testing

As described above, ***eriss*** regained responsibility of the Ranger pre-release toxicity testing program from ERA in 2001–02. Since then, the ***eriss*** ecotoxicology laboratory has undertaken pre-release toxicity testing of Djalkmara Billabong (DjB) water for the 2001–02 and 2002–03 wet seasons, with the results and associated issues described below.

2001–02 pre-release testing

Toxicity testing of DjB water using *M. macleayi* was initiated on 18 December 2001 (Round 1) while additional toxicity testing of DjB water using *M. macleayi* and *H. viridissima* was initiated on 14 January 2002 (Round 2). Tests using *M. mogurnda* could not be undertaken due to a lack of spawning among the brood stock. Round 1 testing produced a LOEC and NOEC of 10% and 3.2% DjB water, respectively. Round 2 testing produced a LOEC and NOEC (from the most sensitive of the two species; *M. macleayi*) of 32% and 10% DjB water, respectively. Some quality issues were associated with the Round 2 *M. macleay*i test (ie delayed test start, question over quality of algal food, high coefficient of variation (CV) in control response), but overall the results were sound and valid.

Although the toxicity test information, data and results were appropriately recorded and filed, it is of concern that the results were not (and are yet to be) written up as a report. Thus, there is no evidence of a recommended minimum dilution rate for release of DjB water into Magela Creek (except for E-mail messages on file). There appears to be a need for clear instruction as to the publication of pre-release toxicity testing results.

2002–03 pre-release testing

The background, results and interpretation of the 2002–03 pre-release toxicity testing program are detailed in Hogan (2003). Toxicity testing of DjB water using *M. macleayi* and *H. viridissima* was initiated on 17–18 December 2002 (Round 1) and again on 9–10 February 2003 (Round 2; duplicate *M. macleayi* tests were run due to concerns about stock culture

health). Due to notification and timing constraints just prior to the Christmas break (ie Round 1 testing), and continued problems with *M. mogurnda* culturing and associated spawning, no fish tests were able to be undertaken. Round 1 testing produced an IC_{50}, LOEC and NOEC (from the most sensitive of the two species; *M. macleayi*) of 1.6%, 0.3% and <0.3% DjB water, respectively. The Round 2 *M. macleayi* tests experienced quality problems (see below), but results from the *H. viridissima* test indicated that DjB water was substantially less toxic than during the Round 1 testing. The *M. macleayi* tests were invalid due to excessive control mortality, with subsequent QA checks indicating the response was most likely due to poor culture health, a problem that has persisted in the laboratory for an extended period and is discussed in more detail below, in *Laboratory Issues*.

As noted above, this testing program was appropriately written up and published as a Supervising Scientist Internal Report (IR 422; Hogan 2003). This report is comprehensive and in addition to providing all the necessary information regarding the pre-release toxicity testing also addressed the problems that were encountered during the testing.

Issues for future pre-release testing

Given that in the next 12 to 18 months DjB will be subsumed by Pit #3 and that RP1 water releases are currently not considered a risk to the downstream environment, it is unclear how long pre-release toxicity testing will continue in the future. In the short term, pre-release toxicity testing of DjB water will still be required for the 2003–04 wet season and possibly for the 2004–05 wet season. In addition, the recommendation by Camilleri et al (2002) that whole effluent toxicity testing be undertaken on overflow reverse osmosis permeate from the final cell of the reconstructed Corridor Creek wetland filter system once it is in operation means that some further pre-release toxicity testing (albeit with a different objective) will be required, probably within the next 2 years. Related to this, the ***oss*** Discussion Paper on ERA's draft application to discharge the RO permeate noted that further whole effluent toxicity testing may be required if the composition of the actual permeate is different to that from the pilot plant permeate which was used for the recent toxicity assessment (see Camilleri et al 2002; and see *Ammonia*, above). Thus, it is most likely that pre-release toxicity programs for Ranger will need to be undertaken for the next 2 to 3 years.

Given this, there is a need for a written agreement between ***eriss*** and ERA that clearly outlines the pre-release toxicity testing process including: the context and objectives of the testing program; the responsibilities of each party; timing of and notification for the commencement of testing in relation to anticipated water discharges; toxicity test details including test species/endpoints, dilution water, statistical treatment of data; reporting requirements and timelines including internal publication; procedures for notification of problems relating to the testing program; and any other special conditions. Essentially, the work needs to be undertaken as if it were a proper contract.

Hogan (2003) also raised a number of other issues of relevance to the pre-release toxicity testing. Problems with stock cultures of *M. mogurnda* and *M. macleayi* compromised the rigour of both the 2001–02 and 2002–03 testing programs, and thus, need to be rectified. Relevant actions have been and are currently underway, and these are dealt with in greater detail below (including recommendations), in *Laboratory Issues*. However, one issue that is worth discussing here is that of the choice of endpoint in the cladoceran (*M. macleayi*) 3-brood test. Hogan (2003) demonstrated that reductions in the number of total offspring per adult were a reflection of adult mortality rather than reproductive impairment (ie smaller brood sizes), suggesting that survival may represent an equally sensitive test endpoint as reproduction. Supporting this, previous research has demonstrated that the two major

contaminants of concern in Ranger waste waters, uranium and $MgSO_4$, elicit the same response by *M.macleayi* as observed in the pre-release testing (Semaan et al 2001, and as summarised by Hogan (2003)). Interestingly, Orchard et al (2002) demonstrated the same response in *M. macleayi* following exposure to copper, but found that cadmium exposure reduced reproductive output rather than simply causing adult death (note that neither of these metals are contaminants of concern in Ranger water waters). Use of survival as the *M. macleayi* test endpoint for pre-release toxicity testing would substantially reduce the time required to undertake the test. Although it appears worthwhile pursuing this issue, Dr Doug Holdway, ARRTC's ecotoxicology expert, strongly discouraged this for the Ranger pre-release toxicity testing regime, largely because the community should have the best possible assurance that the ARR environment is being sufficiently protected. This is a valid and strong argument, and as such, it is not recommended that survival only is measured for *M. macleayi* tests used for assessing the toxicity of Ranger pre-release retention pond waters.

Recommendation

17. That relevant ***eriss*** and ERA staff discuss and document a formal agreement for the conduct of the annual pre-release toxicity testing program. The agreement should clearly outline the pre-release toxicity testing process including the context and objectives of the testing program; the responsibilities of each party; timing of and notification for the commencement of testing in relation to anticipated water discharges; toxicity testing details including test species/endpoints, dilution water and statistical treatment of data; reporting requirements and timelines including internal publication; procedures for notification of problems relating to the testing program; and any other special conditions.

Linking ecotoxicological knowledge and biophysical pathways

ARRTC, in its development of a list of Key Knowledge Needs (KKNs) leading to improved management and protection of the ARR, articulated the following gap in relation to ecotoxicology (ARRTC 2003b):

> The Environmental Research Institute of the Supervising Scientist (ERISS) has accumulated a considerable amount of ecotoxicological knowledge related to the effects of key contaminants on aquatic biota known to be present in the ARR. However, little of this information has been put into a management context, and has not been linked to the various biophysical pathways. For example, what is the importance of the first flush each wet season? Also, we are not aware of work to link contaminanted sediments with biological effects.
>
> Terrestrial environment & food sources have not been investigated. The possible transfer of contaminants such as uranium off of site via the food chain should at least be assessed from a risk assessment perspective. What are possible pathways (eg waterbirds eating fish, sediment-dwelling invertebrates inhabiting RP1 and other close billabongs; terrrestrial animals and birds eating plants and invertebrates inhabiting soil of land-application areas etc).
>
> The mining operation has introduced contaminants into the regional aquatic and terrestrial ecosystems both passively and actively (eg artificial wetlands and irrigation). While a considerable amount of work has been done to 'account' for these contaminants in the major components of the system (eg sediment, groundwater), this still needs to be incorporated into the biophysical models in a transparent way to make the risks (however small) clear to all. There have also been 'uncontrolled' movement of contaminants through trophic pathways both from wetland and irrigation sites (eg pathways such as waterbirds eating fish, sediment-dwelling invertebrates

inhabiting RP1 and other close billabongs; terrrestrial animals and birds eating plants and invertebrates inhabiting soil of land-application areas). The risk assessment framework provides an excellent means of quantifying these issues and provides an essential backup to expert opinion that there is no need for concern.

Consequently, a task has been incorporated into the work program of relevant ***eriss*** staff to review and assess the risks of trophic transfer of contaminants associated with mining in the ARR. This review and assessment needs to be linked to another ARRTC KKN, to develop a conceptual model of the ARR system (including the uranium mines) and reassess and quantify contaminant movement within the biophysical pathways.

As this task is a KKN and therefore considered a necessary component of the work program, there is no further recommendation.

Other ecotoxicology research

In 1994, the functions of ***eriss*** as specified under the *Environment Protection (Alligator Rivers Region) Act 1978* were broadened to include research into the protection and management of wetlands in northern Australia. In fact, the ecotoxicology laboratory research focus had already begun to investigate non-mining related issues (eg herbicide ecotoxicity) by the early 1990s. By 1996, an ecological risk assessment approach was developed with the aim of it being a key component of the ecotoxicology program. van Dam (1998) outlined why and how such an approach was essential for making general toxicity information more useful from an environmental management perspective. Moreover, such changes were seen as an inevitable step towards ensuring the long-term viability and sustainability of the Institute. This section aims to:

- summarise the activities, including research projects and consultancies, of the ecotoxicology program that have not been directly related to mining in the ARR[1]; and
- identify and recommend future key research directions and opportunities that are relevant to tropical aquatic ecosystems.

Alligator Rivers Region

Given ***eriss***'s long assocation with mining activities in the ARR, it is not surprising that much of the non-mining related ecotoxicological research has still focused on the ARR. Since the early 1990s, four non-mining projects with a focus on the ARR were undertaken, as described below. The first two of these, although strictly speaking were carried out as consultancies, are still described because they represent a context for research since undertaken.

Biological toxicity testing of waters from a plunge pool used for recreational purposes (1993; Rippon et al 1998)

This study was plagued with difficulties, and consequently the extent of reporting was limited to a half page summary in the 1992–94 ***eriss*** Annual Research Summary. Little insight was gained on the potential impact of sunscreen lotions on aquatic biota in Kakadu swimming holes.

[1] Note that projects not directly using the ecotoxicology laboratory facilities (eg cane toad risk assessment, *Mimosa pigra* risk assessment, climate change vulnerability assessments) have not been considered in this review.

ANCA consultancy report DN11 – Toxicology of the herbicide AF100 (1993/94; Finlayson et al 1994)

The AF100 herbicide toxicity study was part of a larger project investigating the possible ecological effects arising from the use of herbicides to control *Salvinia molesta* in Kakadu National Park. Given the problems with wetland weeds in northern Australia, herbicide toxicity and risk represents a major issue in the region and one that needs to be further pursued (see tebuthiuron risk assessment, below). As Baird (1996) noted, this internally-published research (Finlayson et al 1994) should have been submitted to a peer-reviewed journal, something that has not occurred to date.

Preliminary assessment of petroleum hydrocarbons in water and sediment at Yellow Water, Kakadu National Park (1997; van Dam et al 1999)

An assessment of petroleum hydrocarbons in the Yellow Water aquatic environment was intiated due to some concerns over the large amount of boating traffic in the area, particularly during the late dry season when flow has ceased and water levels are at their lowest. Although some contamination was detected, the limited funding granted for the project and the timing of the sampling (flow had already commenced; somewhat early than anticipated) meant that it was difficult to gauge the true extent of the issue. Nevertheless, the work was published as a Short Communication in a peer-reviewed journal (van Dam et al 1999).

Assessment of endocrine disruptor activity in Kakadu swimming holes (Hogan et al 2004)

In 2003, a study was initiated to screen for endocrine disruptive (estrogenic) activity in Kakadu swimming holes sampled during peak visitor activity, and to relate levels to effects reported in wildlife. The study was initiated after Dr Mika Peck from the University of Sussex (formerly a PhD student at ***eriss***) alerted Parks Australia North (PAN) and ***eriss*** to recent research that identified some sunscreen ingredients as exhibiting estrogenic activity (ie Schlumpf et al 2001, Schreurs et al 2002, Mueller et al 2003). The project is a collaboration between ***eriss***, PAN and the University of Sussex (Dr Mika Peck). Plunge pool water samples are collected and prepared by ***eriss*** staff and sent to Dr Peck who analyses the samples for endocrine disruptive activity using a yeast estrogen screening assay. Effluent samples from Jabiru sewage treatment ponds and water samples from the downstream receiving environment of Barallil Creek have also be taken to assess another potential source of EDCs in KNP.

Other research

Several other 'non-ARR mining' related projects have been undertaken by the ecotoxicology laboratory, as described below.

Mount Lyell Remediation: Evaluation of rehabilitation options for Mount Lyell using whole-effluent toxicological tests on freshwater organisms (Humphrey et al 1997)

This project comprised part of the Mt Lyell Remediation Research and Demonstration Program, a joint program between the Supervising Scientist and the Tasmanian Department of Environment and Land Management. Whole effluent toxicity (WET) testing was used to estimate the effectiveness of various remediation options for reducing acid drainage from historical copper mining operations at Mt Lyell, Queenstown. Specifically, the aim was to estimate the percentage of acid mine drainage that would be required to be neutralised with lime to produce an effluent mix in which aquatic life could survive. The final results were extremely limited in their usefulness, due largely to difficulties with the test species' intolerance to the naturally soft acidic waters used as test diluent. Nevertheless, a WET testing approach for assessing the effectiveness of waste water treatment/remediation processes can

provide very useful information for environmental management, and could be promoted as an application for mining activities in northern Australia.

Toxicity and ecological risk assessment of the herbicide Tebuthiuron to Australian tropical freshwater organisms (Camilleri et al 1998, van Dam et al 2001)

Herbicide toxicity was one of the first issues identified as an opportunity for the ***eriss*** ecotoxicology laboratory following the broadening of ***eriss***'s functions. The herbicide Tebuthiuron (active ingredient of Graslan®), used to control the woody wetland weed, *Mimosa pigra*, was identified as a potential concern to freshwater communities of the Top End due to its application at the onset of the wet season. Two studies were undertaken: an initial assessment of the toxicity of tebuthiuron to three aquatic species (*M. mogurnda*, *H. viridissima* and *M. macleayi*; Camilleri et al 1998); and an assessment of tebuthiuron toxicity to an alga (*Chlorella* sp.) and aquatic macrophyte (*L. aequinoctialis*) followed by a quantitative ecological risk assessment of tebuthiuron (van Dam et al 2001). A manuscript describing the ecological risk assessment was subsequently rejected (in 2002) by the journal *Environmental Toxicology & Chemistry* due to concerns about the validity of the Exposure Characterisation component of the analysis. The manuscript is in the process of being revised for resubmission. This research represents a model (and a learning experience) for future risk assessments of contaminants by the ***eriss*** ecotoxicology laboratory, and indeed was the basis on which a proposal for risk assessments of two additional herbicides was developed (see *Research Directions & Opportunities*, below).

Copper speciation and toxicity in a contaminated estuary (Eriksen et al 2001a & b).

In 1997, Dr Barbara Nowak from the University of Tasmania successfully applied for funding from ***eriss*** (~$6800) for research following on from from the Mt Lyell Remediation Research and Demonstration Program. Part of the agreement was that ***eriss*** play a role in the project's design and results interpretation and receive co-authorship of any conference and peer-reviewed publications. The project aimed to investigate the relationship between chemical and biological estimates of bioavailability/toxicity of copper in Macquarie Harbour water, which receives contaminated water from the Mt Lyell copper mine. From an output perspective, the project could be considered a success, with two conference presentations and two peer-reviewed publications (Eriksen et al 2001a & b).

External research funding

Track record

It is important to note that none of the above-mentioned research projects have been externally funded. In fact, since 1982, the ***eriss*** ecotoxicology research program has received external funding for only one research project totalling $50 000 in funding. The project, funded by ANZECC and titled 'Validation of metal toxicity data in the ANZECC Water Quality Guidelines using tropical freshwater biota', comprised an Honours and Masters project investigating the influence of various physico-chemical parameters on uranuim toxicity to tropical freshwater biota (Franklin et al 1998, Riethmuller et al 2000), and was summarised above, in *Other uranium toxicity research*.

In the past 2 years there have been two unsuccessful external funding applications for: i) *ecological risk assessments for the herbicides fluroxypyr and metsulfuron*, and ii) *assessing salinity impacts using a risk-based/ecotoxicology approach*. The herbicide risk assessment proposal was part of a larger bid coordinated by the NT Department of Primary Industries and Fisheries to Environment Australia's Weeds of National Significance (WONS) program,

while the salinity ecotoxicology proposal was a collaboration between ***eriss***, University of Technology Sydney, NSW Environment Protection Authority and Sinclair Knight Merz submitted to Land & Water Australia's (LWA) National River Contaminants Program as well as LWA's General Call for Research Proposals. The future of these proposals is discussed below, in *Research Directions & Opportunities*.

Although constrained to some extent by ecotoxicological research related to Ranger and the ARR in general, the lack of externally funded projects within the ecotoxicology program is a serious situation that requires prompt and considerable attention if the program is to be sustainable in the future. Thus, it is imperative that the ecotoxicology program seek out and successfully obtain external funding for at least one major research project within the next 12 months. Recommendations on priority issues on which to focus are provided in the following section.

External funding sources

There exist numerous avenues for obtaining external research funds although it must be acknowledged that ***eriss*** is not always eligible for direct funding. Details of the major funding bodies are provided below, in table 3.

Research directions

Considerations and constraints

With the relocation to Darwin there exist any number of research opportunities for the ecotoxicology laboratory. However, it is important that future directions and opportunities be guided by a number of key criteria/questions as follows, and that these be considered in the context of immediate-term and long-term efforts.

Immediate-term considerations

- Is there a relevant issue for which a funding proposal (draft or unsuccessful) or related proposal already exists and that can be resubmitted for funding with minor changes;
- Does the laboratory possess the existing skills and capacity to address/assess the direction/issue;
- Does the program have the time and resources to undertake the work around existing uranium mining research;
- Does the direction/issue have good potential for external funding from government, industry or elsewhere (eg non-government organisations);
- Does the direction/issue have direct and current relevance to the region's tropical aquatic ecosystems;

Long-term considerations

- What environmental issues currently have or are predicted to have major long-term relevance to the region's tropical aquatic ecosystems;
- Does the direction/issue have good potential for external funding from government, industry or elsewhere (eg non-government organisations); and
- Does the laboratory have the existing skills and capacity or what needs to be done to gain the relevant skills and capacity to address/assess the direction/issue.

Table 3 Details of key funding bodies

Funding type/body	Details	SSD eligibility	Call dates	Web details
Government				
Australian Research Council (ARC)	National Competitive Grants Program (NCGP). Includes: ARC – Disovery (various) ARC – Linkage (various) ARC – Centres & Networks (various)	OSS eligible as partner investigator/ industry partner only Further investigation required	Various – dependent on grant program	www.arc.gov.au
Department of Education, Science and Technology (DEST)	Australian Postgraduate Awards Scheme (APAs) – funded by the Commonwealth but administered through universities.	Co-supervision of postgrad students with university	University-specific, but always around Oct – Nov	www.dest.gov.au/highered/research/apa.htm
Land & Water Australia (LWA)	Various funding programs within 5 R&D arenas. Includes: General Call Research Commissioned Research Postgraduate scholarships	SSD eligible to apply directly for funds	Various – dependent on grant program	www.lwa.gov.au
National Oceans Office (NOO)	No specific R&D programs, but opportunities for research or contract work as part of development of the Northern Regional Marine Plan.	SSD eligible to apply directly for funds or submit tenders	Project-specific	www.oceans.gov.au
AusIndustry	'R&D Start' Program including 'Start Graduate'.	SSD possibly eligible as research partner	Project-specific	www.ausindustry.gov.au
Natural Heritage Trust II (NHT2)	Funding through 'Regional Investments' or 'National/State Investments' under the Trust Programs Rivercare or Coastcare.	Dependent on funding call	Various	www.nht.gov.au
National Action Plan for Salinity and Water Quality (NAP)	21 priority regions affected by salinity & water quality problems are targeted, with focus on development of regional catchment management plans.	Unclear, but presumably eligible if regional needs match SSD expertise	None – access to funds through contact with regional NAP officers.	www.napswq.gov.au
Industry				
Australian Centre for Mining Environmental Research (ACMER)	Research program focuses on providing practical solutions to major environmental issues faced by the minerals industry during operation, decommissioning and closure.	SSD eligible to apply directly for funds	Ongoing	www.acmer.com.au
Australian Coal Association Research Program (ACARP)	Funds projects that lead to improvements in safety standards and performance, a reduction in the environmental impacts of mining and coal utilisation, a reduction in the mine operating cost and technical support to marketing of coal.	SSD eligible to apply directly for funds	Annually, around May	www.acarp.com.au

In other words, more immediately, the ecotoxicology program should look to undertake non-uranium mining research that it has the time and capacity to undertake in addition to the uranium mining research, but in looking ahead, should identify and set out a plan for partnership and capacity building requirements associated with key current/future environmental issues in the region. Of course, the key underlying consideration around all of this is the need to gain external funding. Further, decisions about the short- and long-term directions of the ecotoxicology laboratory need to be considered in the context of the outcomes of a Strategic Review of SSD in progress during 2004.

There appear to be three major constraints associated with the ecotoxicology program undertaking substantial externally funded research: i) lack of a senior ecotoxicologist on staff; ii) commitment to uranium mining research; and iii) ineligibility for various government research funding schemes.

The directive to seek and gain external research funding for the ecotoxicology program, and the associated effort that this requires, necessitates that a senior ecotoxicologist be appointed to lead the ecotoxicology program. It cannot be left to existing technical staff or professional staff from other disciplines with competing demands to successfully gain research funding for ecotoxicology projects. Further, the chances of actually securing funding will be very low if a senior ecotoxicologist is not on staff to act as a Chief/Principal Investigator and project manager. This issue is further addressed below, in *Staffing Issues*.

Depending on the extent to which recommendations regarding the need for further uranium mining ecotoxicological research are adopted, there may be limited time in which to undertake other research. If this situation occurs, a plan needs to be developed that ensures that continual 'background' efforts at developing linkages and concept proposals and targeted capacity building are maintained. For example, summer school, Honours and PhD students can undertake important research that maintains progress on a targeted issue/direction. However, the ability for this to occur again depends on the appointment of a senior ecotoxicologist.

eriss, being a Commonwealth research institute, is ineligible for direct funding under the major Commonwealth Government schemes such as the Australian Research Council's (ARC) National Competitive Grants Program, which includes *ARC – Discovery* and *ARC – Linkage*. ***eriss*** researchers can be listed on ARC funding applications as Partner Investigators, but cannot receive funding from the ARC, which can go only to an eligible higher education institution. Whilst this is an impediment to obtaining funds for research, there are still means and posibilities of having ARC and other externally-funded research carried out at ***eriss***.

Directions and issues

Current (next 12–18 months)

Current directions and issues are discussed in the context of approximately a 12–18 month timeframe, given that most research funding calls for 2004/05 are now closed and there will be limited funding opportunites for this period. The focus on ecological risk assessment or at least risk-based assessment should be maintained where relevant.

Ranger-related research

Based on the outcomes of the review of the Ranger ecotoxicology research it is likely that Ranger-related projects will comprise the majority of the ecotoxicology program's workload for the next 12–18 months. This would also include some level of ecotoxicological research for Jabiluka as described above. Thus, there needs to be a strong commitment by ***eriss*** to providing the necessary resources for this work to be successfully completed and reported/published on.

Test methods and laboratory procedures

In undertaking the above-mentioned Ranger research and also in planning for future research (see below) there will be a need to adapt and refine existing test protocols and potentially commence development of new test protocols (see *Specific opportunities*, below). Further, there is a need to solve various specific problems associated with the ecotoxicology laboratory procedures and equipment, some of which may require specific experimental investigations (see *Laboratory Issues*, below).

Future research planning

Whilst activities to complete the uranium mining ecotoxicology research are underway, there needs to be a continual 'background' effort at developing linkages and concept proposals and targeted capacity building. This effort will be largely guided by the outcomes of the sections directly below on future research directions and specific opportunities. It is critical for a senior ecotoxicologist to be on staff to undertake and coordinate such efforts.

Future (beyong 18 months)

Ecological risk assessment development

It may be beneficial to view future ecotoxicology-related research from a 'process' perspective as well as an 'issue' perspective. Developing and refining formal, quantitative risk assessment models for assessing aquatic environmental issues would provide a foundation on which the ecotoxicology program could progress. The actual development of such models represents a project in its own right, with a particular issue(s) being used as the 'vehicle' to develop and test models. Included in this should be an objective to improve current ecological risk assessment approaches by increasing ecological relevance and better identifying and quantifying variability and uncertainty (eg through Bayesian statistics).

Part of the rationale for this approach is that the tools that are developed have application for other issues and also that the development of a broadly-applicable, scientifically robust process is more likely to attract funding than the assessment of a single issue. This is the approach that Professor Barry Hart (Monash University Water Studies Centre, CRC for Freshwater Ecology) has adopted in recent years with good funding success. It may be possible to link the further development of ecological risk assessment at ***eriss*** with Professor Hart's program. In the event that this approach was to be adopted, it is recommended that the issue(s) proposed to be assessed relate to agricultural/horticultural expansion in the Daly basin and Ord Stage II, and associated use and risks of contaminants including pesticides/herbicides to aquatic ecosystems (see *Specific opportunities*, below).

Mining/point source impacts research

The ecotoxicology program has extensive experience and expertise in the assessment of issues relating to the impact of Ranger waste waters and associated toxicants on downstream freshwater ecosystems. It would seem commonsense that this capability be further utilised and research opportunities pursued in the mining area (including operational, abandoned, closed and proposed mines) or any area for which mining and other point source contaminant discharges are an issue. This should focus on the application of risk assessment approaches for assessing mining and other point source impacts consistent with the ANZECC and ARMCANZ (2000) Water Quality Guidelines and the subsequent Australian Centre for Mining Environmental Research (ACMER) publication (ACMER 2003).

Telephone conversations and meetings to discuss ecotoxicology opportunities have been held with Northern Territory Government officials, namely Pamela Sanders (Senior Project Manager, Mines & Petroleum Management Division) of the Department of Business, Industry

& Resource Development (DBIRD) and Michael Lawton (Director, Water Quality) of the Department of Infrastructure, Planning & Environment (DIPE), and Associate Professor David Parry of Charles Darwin University (CDU). There is definite interest from all the above-mentioned parties for collaborative research (see *Specific opportunities*, below) and possibly commercial work (see *Commercial opportunities*, below).

Opportunities in this area may also exist in Queensland and Western Australia. Whilst limited discussions have been held as part of this review, it is strongly recommended that opportunities in these regions be further explored. Key agencies for these regions include the Queensland Environment Protection Agency and Department of Natural Resources and Mines, and the Western Australian Department of Environment Protection and Department of Conservation and Land Management. Relevant activities in these regions for which R&D would be beneficial include mining and waste water treatment.

Marine ecotoxicology

eriss's relocation to Darwin has provided an opportunity to at least consider the possibility of developing a marine ecotoxicology capability. Current economic growth and projected urban and industrial development is beginning to place emphasis on the need for proper environmental management to minimise impacts on the environment. This is being recognised to some extent, with the recent development of the *Darwin Harbour Regional Plan of Management* (Darwin Harbour Advisory Committee 2003). The Plan recognises that Darwin Harbour water quality can be impacted by pollution from several sources within the region, including:

- Primary and secondary treated sewage is discharged from treatment plants at Leanyer (into Buffalo Creek), Palmerston (into Myrmidon Creek), Berrimah (into Bleesers Creek) and Ludmilla (into Ludmilla Creek), with macerated and disinfected sewage from Larrakeyah released from the deepwater outfall into the harbour;
- Ludmilla treatment plant currently does not have the capacity to deal with large stormwater inputs in the wet season. At times of peak flow, some sewer flows bypass the treatment plant and are released directly into waterways;
- Stormwater from throughout the region washes contaminants, nutrients and sediments from various land uses into the harbour;
- Runoff from agriculture and horticulture can include fertilisers (nutrients), pesticides (contaminants) and sediments;
- Currently, industrial trade wastes are discharged to the sewer system, prior to discharge to waterways with other treated sewage;
- Leachate from waste disposal sites, land fills and reclamation sites can be high in contaminants;
- Sediments and associated contaminants are dispersed during dredging and disposal of dredge spoil;
- Release of contaminants, nutrients and sediments can occur through accidental product spills when ship loading or during transport;
- Erosion and sedimentation commonly occur as a consequence of land clearing for development, as well as other changes in catchment hydrology and harbour hydrodynamics;
- Reclamation and development in low-lying coastal areas can lead to acid leachate from acid sulfate soils (Darwin Harbour Advisory Committee 2003).

Many of the NT Government's Major Projects (see http://notes.nt.gov.au/dbird/majorproj.nsf) have some connection with the coastal or marine environment. Associated with these projects are numerous environmental issues that will most likely have major long-term relevance to the region's coastal and marine ecosystems. Thus, there appears to exist numerous oppportunities ***eriss*** to undertake marine ecotoxicological research in the region. Table 4 lists major projects/activities in the Top End that have the potential to impact on coastal or marine ecosystems and the types of ecotoxicological assessments that could be undertaken. For opportunities in these areas to be further investigated and hopefully realised, contact would need to be made with the relevant NT Government departments (as listed in table 4).

As an example of the type of opportunity, recommendations arising from the Environment Assessment Report (EAR) for the Wickham Point 10 million tonnes per annum (10 MTPA) Liquified Natural Gas (LNG) plant (DIPE 2002) included the following:

> If chemical additives used in hydrotest water pose a **risk of toxicity to marine life** in the Harbour, the proponent will require a Waste Discharge Licence. The Licence will require the proponent to analyse the hydrotest formulation to be used (**to assess the potential toxicity to marine biota**) and to monitor the receiving water to ensure adequate dilution and dispersion to reduce toxicity to an acceptable level…

and

> Oil spill contingency plans for the construction dock and product loading jetty shall be prepared by the proponent … The site-specific plan shall include
>
> - An assessment of potential risks of spills of credible volumes;…

A marine ecotoxicology capability would enable ***eriss*** to work with industry and NT Government, either in a collaborative research or commercial capacity (see *Commercial Ecotoxicology Projects*, below), to address such issues. Discussions have already been initiated with Mr Michael Lawton of DIPE regarding this issue. Given the early stages of the gas fields from which the LNG is to be derived, the toxicity assessments recommended in the EAR are still at least 2 years off, and thus, depending on agreed research priorities over the next 2 years, may represent an opportunity for ***eriss***.

The development of a marine ecotoxicology capability should be seen as a long-term goal, perhaps with a 5 year timeframe (unless sooner major opportunties dictate otherwise). The ***eriss*** ecotoxicology laboratory possesses much of the necessary infrastructure to undertake marine toxicity testing, although there would need to be some additional equipment purchased (eg small, ~1000 L seawater storage tank, filter, test equipment). If possible, a marine ecotoxicology capability should be linked to CDU, where the analytical/environmental chemistry laboratories (run by Associate Professor David Parry) and a marine aquaculture facility, including a continuous salt water supply, are located. Linkages to David Parry's research program would enable a risk assessment approach to be adopted for marine projects, while use of the aquaculture facility would boost the capability for marine test development (for further details, see *Specific opportunities*, below). It should also be noted that the Australian Institute of Marine Sciences (AIMS) is currently establishing a presence in Darwin, through a partnership with the Australian National University (ANU) as the Arafura Timor Research Facility, and opportunities to collaborate on the above-mentioned issues may exist. Additionally, there is no need to restrict marine research opportunities to the Northern Territory, and the ***eriss*** Ecotoxicology program should be open to partnerships/collaborations with other groups including the Great Barrier Reef Marine Park Authority (GBRMPA), CSIRO and relevant universities (eg James Cook University, JCU; Uinversity of Queensland/National Research Centre for Environmental Toxicology, NRCET).

Table 4 Developments and activities in the Northern Territory associated with the coastal and marine environment for which opportunities might exist for ecotoxicological research

Project/Activity	Issues	Types of ecotoxicological studies	Responsible NT Government Dept. & contact
Oil and Gas Major Projects *eg Greater Sunrise gas field* *Evans Shoal gas field* *Phillips LNG plant*	Opening of new oil and gas fields Construction of onshore/offshore gas and methanol processing plants	WET testing of produced formation waters (PFWs) Ecotoxicology of crude oils, gas condensates	Department of Business, Industry & Resource Development (DBIRD) (http://www.dbird.nt.gov.au/) *Petroleum Environment Advisor:* Mr Chris Amey (Tel. 8999 6826) Department of Infrastructure, Planning & Environment (DIPE) (http://www.ipe.nt.gov.au/) *Director, Water Quality:* Michael Lawton (Tel. 8924 4031)
East Arm Wharf Development	Bulk liquids berth Loading/unloading of hazardous liquids (eg oil) Channel dredging	Ecotoxicology of hazardous liquids Elutriate toxicity testing of dredged sediments	DIPE *Manager, Environmental Assessment:* Helge Pedersen (Tel. 8924 4138) Janice Warren Michael Lawton
Darwin City Waterfront Redevelopment	Dredging Runoff	Elutriate toxicity testing of dredged sediments Ecotoxicology of urban/stormwater runoff	DIPE *Assessment Officer:* Rod Johnson (Tel. 8924 4139) *Contaminated Sites:* Robyn Henderson (Tel. 8924 4139)
Ludmilla Sewage Treatment Plant	Primary treated sewage effluent discharge	WET testing of STP effluent Assessment of effectiveness of treatment upgrades Assessment of endocrine disruptive activity	Power & Water Authority (http://www.nt.gov.au/powerwater/) *Waste Water Quality:* Andrew Mills (Tel. 8924 7096)
General urban/industrial activity & development	Urban/stormwater runoff into waterways	Ecotoxicology of urban/stormwater runoff	DIPE Michael Lawton Armando Padovan (Tel. 8924 4139) Darwin City Council (www.darcity.nt.gov.au) Dave Thiele (Tel. 8982 2511)
Mining operations *eg GEMCO, Groote Eylandt* *Alcan, Gove* *MIM, McArthur River*	Mine waste water discharges Ore/product loading and off-loading facilities	WET testing of mine waste waters Ecotoxicology of relevant toxicants	DBIRD *Manager, Environment & Policy:* Mr Steve Tatzenko (Tel. 8999 5372) *Senior Mines Project Manager:* Pamela Sanders (Tel. 8999 5162) DIPE Michael Lawton

Provision of training

The wetland research/NCTWR component of ***eriss*** has always had a commitment to providing training in wetland research and management, both nationally and internationally. Ecotoxicology and risk assessment has been a component of the majority of training programs/study tours ***eriss*** has been involved with. The Ecotoxicology Program should continue to undertake such activities, with a view to:

- providing formal training as part of Australian Higher Education courses, through lecturing and formal incorporation of ecotoxicology components in courses (possibly facilitated via NCTWR); and
- looking to develop training/capacity-building activities in tropical countries, probably focusing on south-east Asia.

Concept of an Ecotoxicology Consortium

In 2001, the concept of a 'Northern Australian Ecotoxicology Centre' was flagged with SSD (Arthur Johnston and Rick van Dam) by George Lukacs of the Australian Centre for Tropical Freshwater Research (ACTFR) at James Cook University, a partner with ***eriss*** in the NCTWR. In early 2002, some preliminary notes were drafted outlining a possible framework for the scope, structure and administration of such a centre (Appendix F). Although the concept has progressed no further, it retains much relevance for ***eriss***, particularly in terms of its long-term future. Thus, linkages and collaborations with other institutions should be considered in the context of the potential establishment of a Northern Australian or National Ecotoxicology Consortium/Centre Finally, with SSD currently undergoing an extensive internal strategic review to identify appropriate possibilities for and help guide its long-term future structure and function, it would be worthwhile for the concept of the ecotoxicology consortium to be identified within the strategic review as a significant opportunity for ***eriss***. This will help ensure that any further discussions and decisions on the issue will be consistent with the strategic direction of the Division.

Recommendations

18. That ecotoxicology research directions over the next 12 to 18 months focus on:
    - Ranger (and Jabiluka) related research;
    - Strategically focused improvement and development of test methods and laboratory procedures; and
    - Future strategic research planning efforts.
19. That future ecotoxicology research directions (ie beyond 18 months) focus on:
    - Ecological risk assessment development;
    - Mining/point source impacts research across northern Australia;
    - Developing a marine ecotoxicology capability (~5 year timeframe); and
    - Provision of training, both nationally and internationally.
20. That linkages and collaborations with other institutions are considered in the context of the possible establishment of a Northern Australian or National Ecotoxicology Consortium/Centre, and that the concept of the consortium to be identified within the strategic review as a significant opportunity for ***eriss***.

Specific opportunities

Previous funding proposals

There currently exists previously unsuccessful research project/funding proposals for the following two projects:

- Ecological risk assessment of the herbicides fluroxypyr and metsulfuron methyl following application to *Mimosa pigra*; and
- A risk-based approach to salinity toxicity for inland aquatic ecosystems.

It is highly recommended that the herbicide risk assessment proposal be revised and submitted for an *ARC – Linkage* or *ARC – Discovery* (or other as appropriate) Grant in 2004. The proposal will again require the involvement of CDU, through Associate Professor David Parry, and the other project partners (NT Govt Weeds Branch, Lower Mary River Landcare Group, Melaleuca Station). In particular, the status of the herbicide spraying program on Melaleuca Station needs to be ascertained.

To improve the chances of gaining funding, it may be worthwhile shifting the project focus from the issue of herbicides to the development of a relevant risk assessment model for tropical aquatic ecosystems, and considering a collaboration with Professor Hart's research group at Monash University. As mentioned above, his program has been successful in obtaining funding to develop formal risk assessment models. This idea requires further internal discussion and discussion with Barry Hart.

The salinity ecotoxicology proposal, being a large collaboration coordinated mostly through UTS and NSW EPA, is more difficult for ***eriss*** to resubmit for funding. Current efforts at determining whether the original project team is willing to find another funding body for which to submit the proposal should be maintained.

Influence of DOM on the toxicity of uranium (and other metals) to tropical freshwater biota

As noted above, DOM is the least studied physico-chemical variable with regards to uranium bioavailability and toxicity yet may well be the most influential. Although not considered essential to completion of the uranium mining ecotoxicology research program, there seems more than sufficient justification to investigate it through a student project. Somewhat related to this, sorption (to insoluble organic matter) also is known to play a dominant role in determining the fate of uranium in freshwater systems, but bioavailability and toxicity of sorbed uranium has not been studied (ANZECC & ARMCANZ 2000). Thus, a postgraduate project, most probably a PhD, could be initiated that investigates and attempts to quantify the influence of the above two physico-chemical variables on the bioavailability and toxicity of uranium to a range of species (note that this has been listed as Recommendation 4, in the *Status of Ranger Ecotoxicology Research* section).

Impacts/risks of mining activities on the Gulf of Carpentaria

An opportunity may arise within the next 6 months to obtain funding through the National Oceans Office (NOO) for integrated ecological risk assessments of active mines in the Gulf of Carpentaria region (Bamaga to Nhulunbuy) (see *Specific opportunities*, below). However, this opportunity may require a marine ecotoxicology capability, which almost certainly could not be developed within the above timeframe. Nevertheless, it is possible that any ecotoxicology component could be contracted out, with the rest of the assessment being done by the ***eriss*** Ecological Risk Assessment program. Therefore, contact with NOO should be maintained in order to maximise the potential for future opportunities.

Development of a collaborative research project on mining and water quality

Numerous mining operations in the Top End have water management systems that include discharge of mine waters to the local aquatic environment. According to DBIRD (through Pamela Sanders), in most instances, the mines are operated by small companies and/or are in care and maintenance mode, and resources for commercial water quality assessment and monitoring are not available. Further, given the high wet season rainfall, controlled waste water discharges are often a necessity in order to minimise risks of uncontrolled discharges (ie over-topping or failure of waste water/tailings ponds), apparently regardless of the level of contamination and potential for toxicity of the waters and impacts to the downstream biota. In short, the message (coming from DBIRD) is that mining companies have insufficient funds to undertake toxicity assessments, and that toxicity testing programs do not provide practical/useable results for effective water systems management of many mining operations in the NT. However, this argument, whilst understandable from one perspective, should not be used as a basis for allowing an operation or care and maintenance of a mining operation in a manner that may not be ecologically sustainable. Thus, a way must be found to move forward.

DIPE (through Michael Lawton) has expressed concern over the waste water management of numerous Top End mines but, like DBIRD, acknowledges the limited financial ability of the operators to appropriately address this. Two areas identified by DIPE are:

- knowing more about regionally-relevant toxicants (eg trigger values geared for tropical freshwater ecosystems) and better understanding of the importance of local physico-chemical variables; and
- understanding the significance and impact of intermittent wet season overflows of mine process waters from small, under-resourced operations and operations in 'close-out' mode, and what constitutes an (un)acceptable impact?

An option currently being discussed between ***eriss***, DBIRD, DIPE and CDU is to apply for collaborative research funding to address these issues. Based on discussions to date, one approach to a study could be to use several mining operations as 'case studies' for:

- determining the actual long-term impacts and risks of mining discharges in the context of the management systems required to cope with the highly seasonal wet-dry tropical climate (ie ephemeral water bodies, first flush issues, etc);
- developing a more relevant framework or model for toxicity assessment (and possibly monitoring) of discharge/pond waters from mining operations in the Top End.

Such a project could also address relative risks of regular controlled releases versus infrequent uncontrolled discharges (ie over-topping or failure of waste water/tailings ponds) and/or could focus on small, under-resourced operations and/or operations in closure phase. It is imperative that discussions continue towards a final proposal for funding, with a view to submitting the proposal in 2004. To ensure this happens, the next step needs to be a workshop with the above-mentioned groups to determine priority issues and agree on a strategic research topic.

Endocrine disrupting compounds (EDCs)

The ecotoxicology laboratory is currently involved in a small collaborative project with Dr Mika Peck (University of Sussex) screening for endocrine disruptive activity in Kakadu plunge pools and Jabiru township sewage effluent and receiving water. The current project, although small, is excellent from an environmental, collaborative and technical point of view and could lead to further research and, potentially, funding opportunities. Recent correspondence with Dr Peck has indicated that this type of research could be extended to other contaminant issues in the Top End, including sewage treatment plant (STP) effluents

and even mining effluents. Further, there may be opportunity for the ***eriss*** ecotoxicology laboratory to develop the capacity to undertake the relevant analyses.

EDCs are of major concern worldwide, and research on identification and effects of EDCs is is still high priority. There is likely to exist little information on EDCs in tropical aquatic ecosystems and this might be considered an important area of investigation. Dr Louis Tremblay (Centre for Environmental Toxicology – CENTOX, Lincoln University) and Associate Professor Richard Lim (University of Technology Sydney – UTS), EDC experts from New Zealand and Australia, respectively, have recently expressed interest in extending their Australian collaborative efforts to working with ***eriss***. Their reputations in the field would boost chances of gaining external funding.

Development of ecotoxicological tests for tropical marine species

A number of presentations at the recent SETAC/ASE Asia Pacific 2003 ecotoxicology and environmental chemistry conference (28 September – 1 October 2003) highlighted the lack of ecotoxicological data for tropical species and the heavy reliance on temperate species data for ecological risk assessment or deriving guidelines/criteria for protecting tropical aquatic ecosystems. This will become a significant issue over the next few years for Top End coastal waters, which will be subjected to comparatively high development pressure. Consequently, there may be an opportunity to gain funding for the development of appropriate methodologies for assessing contaminant risks and impacts to tropical marine species.

Toxicity tests for several marine species could be developed at ***eriss*** and in conjunction with CDU (and possibly others). For example, documented toxicity test protocols exist for several marine species that occur in the tropics including the diatom, *Nitzschia closterium*, the red-brown alga, *Isochrysis* sp., the tiger prawn, *Penaeus monodon*, and barramundi (*Lates calcarifer*). All these species are currently being cultured (or cultures maintained) at the aquaculture facility at CDU (D Parry, CDU, pers comm). In addition, tests exist for temperate species for which related species are likely to occur in the tropics (eg sydney rock oyster, *Saccostrea commercialis*). Assuming adequate staff resources, the *Nitzschia* and *Isochrsysis* tests could be implemented in the laboratory without great difficulty. It should be noted that Alicia Hogan, an EA-4 level Research Officer in the ecotoxicology program, has direct experience in conducting the *Nitzschia* test and is very enthusiastic about adding it to the capabilities of the ecotoxicology laboratory.

An initial funding proposal on or relating to the development of the above capability should be completed by mid-2005. It is likely that the project would include at least one PhD student and possibly a post-doctoral position. It will be necessary to communicate with other research groups developing tropical marine toxicity tests (eg NRCET have developed toxicity testing methods using corals) and ensure that the proposal complements rather than duplicates existing efforts, and possibly includes the relevant research groups as collaborators. If more appropriate, the project could be linked to an issue (as described above for the development of ecological risk assessment models), such as oil and gas development in the region or even EDCs/stormwater/sewage effluent.

Other opportunities

A number of additional research topics or issues that may represent promising funding opportunities in the future are listed below. Whilst no direct action is currently required towards developing concept proposals, it would be worthwhile to keep a 'watching brief' on the topics/issues in order to be in a position to identify if and when they could/should be further developed.

- *Laser ablation ICPMS techniques:* a new instrument/technique recently acquired by CDU and in which ***eriss*** has a partial stake, that can directly measure metal concentrations in hard tissues. Among other things, it has been proposed as a useful tool for environmental monitoring using organisms such as corals and fish.
- *Ord Stage II development:* a proposed major irrigated agriculture development on the Western Australia – Northern Territory border (Keep River) for which there will be numerous environmental contamination issues (eg pesticides, metals, salinity, nutrients).
- *Daly basin development:* areas of newly-developed intensive agriculture/horticulture activities through the Daly basin for which there may be environmental contamination issues (eg pesticides).
- *Further freshwater ecotoxicology development:* there exist several additional freshwater toxicity tests that could be developed to full protocol stage by student or other funded projects (eg black-striped and chequered rainbowfish, *Azolla pinnata*, hornwort, *Ceratophyllum demersum*).

Recommendations

21. That the fluroxypyr and metsulfuron methyl risk assessment proposal be revised as suggested in the report text and submitted for an *ARC – Linkage* or *ARC – Discovery* Grant in 2004.
22. That efforts to resubmit the ***eriss***/UTS/NSW-EPA/SKM salinity ecotoxicology/risk assessment proposal are kept active.
23. That contact be maintained with the National Oceans Office (NOO), primarily Dr Ilse Kiessling, in order to maximise the potential for future opportunities.
24. That discussions continue towards a collaborative research proposal for developing an appropriate framework or model for assessing risks and impacts of mine water discharges on freshwater ecosystems in tropical Australia, with a view to submitting for funding in 2004. The next step needs to be a group workshop to discuss priority issues and agree on a strategic research theme.
25. That the prospect of developing a research project proposal based on endocrine disrupting compounds (EDCs) in tropical aquatic ecosystems be further investigated, with discussions continued with Dr Mika Peck, Dr Louis Tremblay and Associate Professor Richard Lim.
26. That a funding proposal be completed by mid-2005 for the development (or first stage of development) of appropriate methodologies for assessing contaminant risks and impacts to tropical marine species.

Commercial ecotoxicology

Track record

Since 1993, the ***eriss*** ecotoxicology research program has undertaken 8 commercial projects totalling approximately $44 000 in funding. Table 5 lists the projects and the amounts and sources of funding.

Given the infrequent nature of the commercial work undertaken in the past by the laboratory, there has been little in the way of developing formal and accurate costing estimates as well as

consideration of other issues relating to such work including the use of contracts/letters of agreement, standard quoting and reporting templates, public liability and professional indemnity insurance, project management, and specific QA/QC procedures that are now expected by clients. These issues are not discussed in detail here but rather expected to be elaborated upon in a separate discussion paper on the commercial viability/potential of ***eriss*** ecotoxicology laboratory.

A consultancy that is not listed in table 5, because it was largely *gratis* in nature, was an assessment of the toxicity of retention pond water toxicity from an abandoned minesite, undertaken in late 2001 on behalf of the NT Government. Without specifying details or apportioning blame, this piece of work represents the worst-case-scenario for a commercial project. No formal contract or Letter of Agreement was developed, information and communication management was poor, and consequently, the parties' roles and responsibilities, the aims and scope of work, and the timing and reporting requirements were poorly defined or not defined at all. The final report was eventually submitted to the client in mid 2003, unacceptably late, and was agreed by both parties as being inappropriate for official use or release. Perhaps the only positive outcome of this project was that it now stands as a clear example of how not to undertake a commercial project.

Table 5 List of commercial projects undertaken by *eriss* ecotoxicology laboratory (1993–2003)

Year(s)	Project	Client	Amount ($)
2003	Chronic toxicity testing of a nickel mine tailings liquor to the tropical green hydra, *Hydra viridissima*, and the duckweed, *Lemna aequinoctialis*	CSIRO Centre for Advanced Analytical Chemistry	3500
2001–2002	Biological toxicity testing of water from the Ranger Uranium Mine Process Water Treatment Pilot Plant	EWL Sciences Pty Ltd	4700
2000	Acute toxicity of nickel tailings liquor to the tropical freshwater cladoceran, *Moinodaphnia macleayi*, and purple spotted gudgeon, *Mogurnda mogurnda*	CSIRO Centre for Advanced Analytical Chemistry	4660
1999–2000	Comments on the ecological risk assessment component of the final EIS for extensions to the irrigated cotton development on Pillicawarrina	NSW Departmnent of Land and Water Conservation	8500
1998–1999	Toxicity assessment of pit water at Tom's Gully Gold Mine for the 1998–1999 wet season	Sirocco Resources NL	4000
1996	Acute toxicity of kiln grade spar (KGS) leachate to sac-fry of the purple-spotted gudgeon, *Mogurnda mogurnda*	CSIRO Centre for Advanced Analytical Chemistry	4000
1993/94	ANCA consultancy report DN11 – Toxicology of the herbicide AF100	Australian Nature Conservation Agency	9800
1993	Biological toxicity testing of waters from a plunge pool used for recreational purposes	Australian Nature & Conservation Agency	5200
		Total funding from commercial projects	**$44 360**

The Northern Tropical/Sub-Tropical Region

For the purposes of this review, the area of northern Australia in which ***eriss***'s tropical freshwater ecotoxicology capability is considered relevant corresponds to Australia's equatorial, tropical and (eastern) sub-tropical regions, as defined by Stern et al (2003; http://www.bom.gov.au/climate/environ/other/koppen_explain.shtml) using a modified Köppen classification system (figure 2). Broadly, the region extends from Broome in WA, across the Top End, coastal Gulf Country and Cape York, and through east and south-east

Qld. Amongst many criteria, the region's characteristics include an average annual rainfall above 600 mm, the majority of which is spread over 4–7 months, and an average annual temperature of above 18°C.

For the marine environment, the area of relevance extends north from the Gulf of Learmonth in WA (ie approx. Latitude 22 S), across northern Australia and southward along the Qld coast to Mackay (approx. Latitude 21 S) (figure 3). This region is characterised by warm marine waters, with average sea surface temperatures above 25°C (Australian Oceanographic Data Centre 2003; http://www.aodc.gov.au/), and thus, is relevant to the likely future tropical marine research and commercial directions of the ***eriss*** ecotoxicology laboratory.

The market in northern Australia

There appear to exist substantial opportunities for commercial ecotoxicology across northern Australia. However, from personal experience as Director of the commercial ecotoxicology laboratory at SKM from mid-2001 to early-2003, opportunities or prospects are not readily progressed to contract. In most cases, the probability of a prospect becoming a proposal, let alone a project, is highly dependent on state legislation and associated licencing; industries will usually only undertake/pay for ecotoxicology if it is a licence requirement. Currently, the recommendations within various national guideline documents (eg *Australian and New Zealand Guidelines for Fresh and Marine Water Quality* – ANZECC & ARMCANZ 2000, *National Ocean Disposal Guidelines for Dredged Material* – Environment Australia 2002) for the application of types of biological assessment are generally insufficient to convince industries to adopt such approaches. Both the Queensland and Western Australian governments are in the early stages of incorporating the application of biological assessment into licences for various industries/activities. In contrast, the Northern Territory is reluctant to incorporate such measures into licences, it seems, due to a perceived lack of need or relevance for the requirement of such approaches and the financial constraints it might place on the licencee. Thus, relative to the opportunities that can be identified, the actual size of the existing market in northern Australia is relatively small.

Despite the above summation of the existing ecotoxicology market, based on personal experience and recent discussions with several environmental consultants, there is still some commercial ecotoxicology being undertaken for activities/industries in northern Australia. The bulk of these industries/activities occur in or discharge waste into the marine environment (eg oil and gas industry, sewage treatment plant ocean discharges, dredging operations), an area where ***eriss*** currently has no ecotoxicological expertise. Much less commercial ecotoxicological work is being done for tropical freshwater issues. However, this may simply be due to the fact that with the exception of the the ***eriss*** ecotoxicology laboratory, no Australian facility has a tropical freshwater ecotoxicology capability (NB – CSIRO's Centre for Advanced Analytical Chemistry has a tropical algal species that it uses for commercial work). If the freshwater tropical market can be established, then ***eriss*** will have no direct competitors, at least for the time being.

Industries/sectors across northern Australia that may have a need/requirement to utilise freshwater ecotoxicology services include:

- Mining and minerals processing;
- Water (eg sewage treatment plants);
- Horticulture (eg fruit and vegetables);

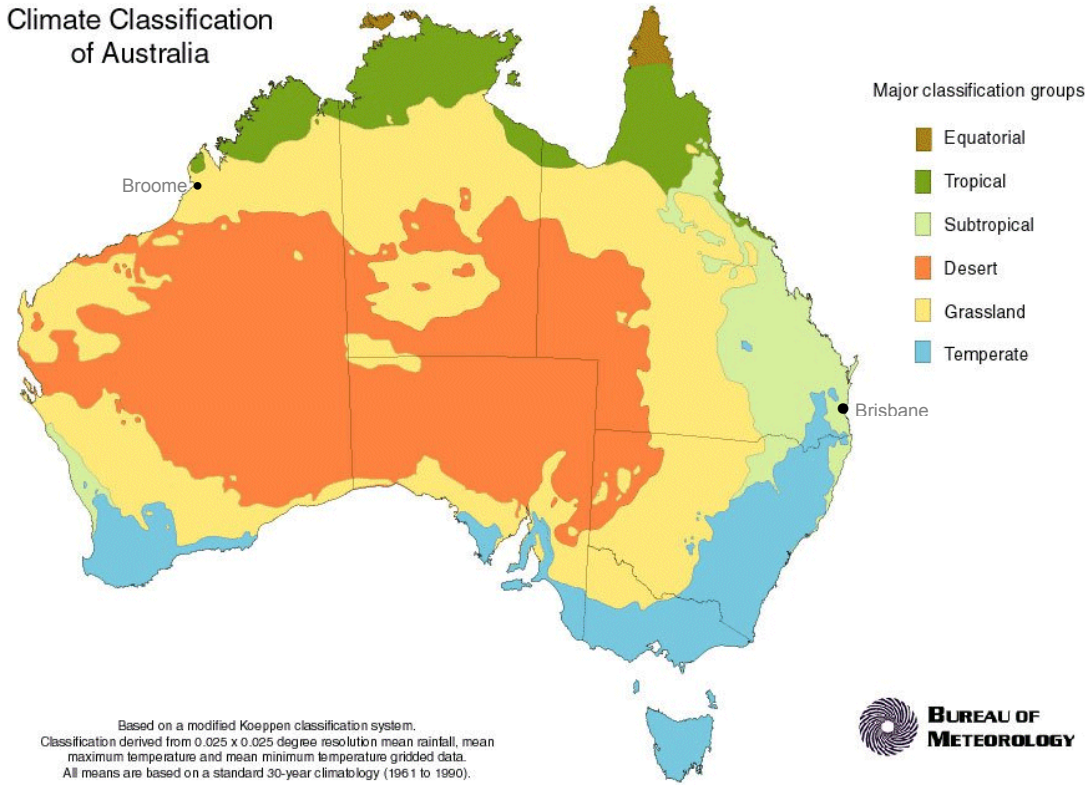

Figure 2 Map of Australia showing the major climate classifications (from Stern et al 2003)

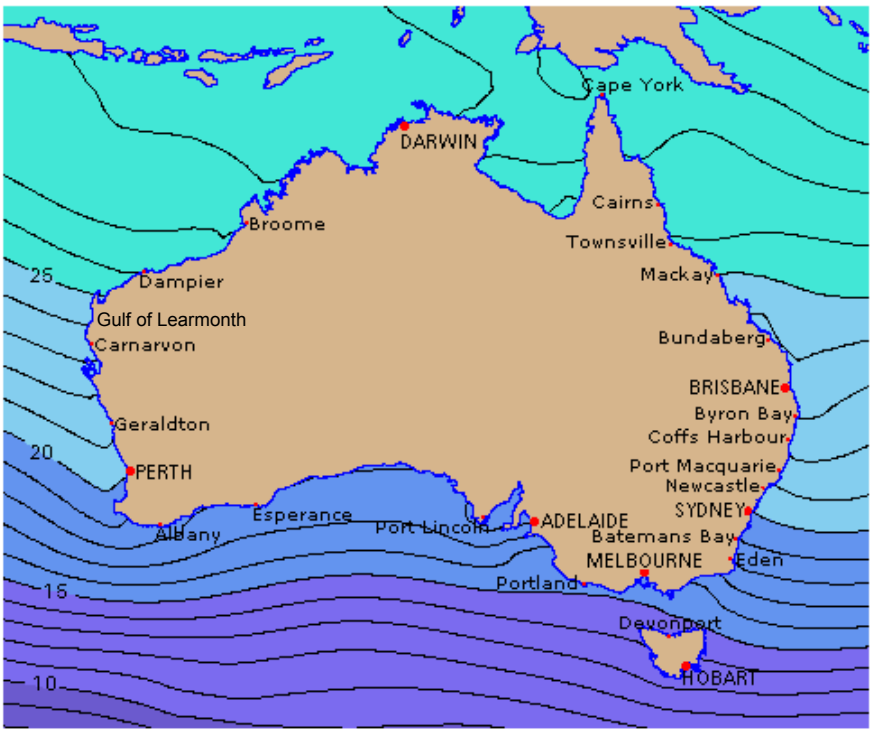

Figure 3 Map of Australia showing average surface sea temperatures (from Australian Oceanographic Data Centre 2003)

- Agriculture (eg broad acre cropping, including cotton and sugar cane);
- State government;
- Local government and town councils.

The other major industry in the north is tourism. Whilst it is unlikely that there will be major opportunities in this area, there may exist potential for ecotoxicological work in areas of ecological/conservation importance. Aquaculture is another growing industry in northern Australia, and one where there may be future opportunities for commercial ecotoxicology.

Industries/sectors associated with the marine environment, for which ***eriss*** might look to target in the future include:

- Mining and minerals processing;
- Oil and gas;
- Aquaculture;
- State government;
- Local government.

Prospective clients

With an understanding of the relevant industries/activities, prospective clients can be identified. Both the government and private sectors should be seen as potential sources of commercial ecotoxicology work.

Government

Relevant government departments/agencies are listed in table 6. The list represents departments or Authorities/Corporations that in some way regulate, oversee or manage particular industries or activities. Some may not represent prospective clients, but rather important points of contact for identifying prospective clients and associated prospects. Others, such as the numerous Port Authorities/Corporations and local governments do represent prospective clients. Other semi-government bodies such as some of the Water Authorities are listed and discussed below as industries.

Industry

This section is intended to provide an indicative rather than exhaustive list of potential industry clients across northern Australia. It is expected that a more comprehensive list would be developed at a later date as part of a concerted business development campaign. Table 7 provides an indication of the potential industry clients in the Northern Territory, covering both freshwater and marine issues. It is evident that mining is by far the major relevant industry, followed by the oil and gas industry. Although ecotoxicology is utilised by numerous water authorities/corporations in south-eastern Australia for assessing the toxicity of STP effluents (eg Sydney Water, Melbourne Water, Hunter Water), the overall lack of urban and industrial development in northern Australia will limit prospects with such clients, execpt perhaps some Queensland urban centres such as Cairns, Townsville and Rockhampton (see below).

In north-west Western Australia it is likely that mining and petroleum companies and port authorities will comprise the majority of prospective clients. These would include Argyle Diamond Mine, Woodside Petroleum and BHP. Horticulture and intensive agriculture activities around Kununurra might represent opportunities although prospective clients have not been identified at this stage.

In Queensland, there exist numerous mining operations and mineral resources in sub-tropical/tropical environments, with some of the larger operating companies including Xstrata Plc, Newcrest Mining Ltd, Zinifex Century Mine Ltd, Comalco Ltd and Southern Pacific Petroleum Pty Ltd. In addition, local councils of larger urban centres (eg Townsville, Cairns, Rockhampton, Mackay) have responsibility for sewage treatment and thus, depending on Qld EPA licence requirements, represent prospective clients. Horticulture and intensive agriculture activities throughout tropical Qld might represent opportunities although prospective clients have not been identified at this stage.

Table 6 Government departments/agencies/authorities relevant to industries or activities that may require ecotoxicology services

Jurisdiction	Department/Agency
Commonwealth	Department of the Environment and Heritage (DEH)
	Australian Pesticides & Veterinary Medicines Authority (APVMA) – *for registration of agricultural chemical products and veterinary chemical products*
	National Industrial Chemicals Notification & Assessment Scheme (NICNAS) – *for registration of industrial chemicals*
	National Oceans Office (NOO)
	Department of Industry, Tourism and Resources (DITR)
	Department of Agriculture, Fisheries and Forestry (AFFA)
Northern Territory	Department of Infrastructure, Planning and Environment (DIPE)
	Department of Business, Industry and Resource Development (DBIRD)
	Department of the Chief Minister (Office of Territory Development)
	Darwin Port Corporation
Queensland	Environment Protection Agency
	Department of Natural Resources and Mines
	Department of Primary Industries
	Department of State Development
	Port Authorities and Corporations (includes Port of Brisbane Corporation, Ports Corporation Queensland, Gladstone Port Authority, Cairns Port Authority and Port of Townsville)
Western Australia	Department of Environmental Protection (DEP)
	Environmental Protection Authority
	Department of Agriculture
	Department of Industry and Resources (DIR)
	Department of Conservation and Land Management (CALM)
	Water and Rivers Commission
	Port Authorities (includes Dampier, Broome and Port Hedland Port Authorities)
Local	*NT:* Darwin City Council (DCC), Palmerston CC, Litchfield Shire Council, Katherine Town Council, Tennant Creek Town Council
	WA: eg Shire of Wyndham-East Kimberley, Shire of Derby-West Kimberley, Shire of Broome, Town of Port Hedland

Environmental consultantants

Environmental consulting firms also represent a potential source of commercial ecotoxicology work. Consulting firms can sub-contract the ***eriss*** ecotoxicology laboratory to undertake ecotoxicological investigations that form part of larger assessments. This is the manner in which the CSIRO Centre for Advanced Analytical Chemistry has in the past provided commercial work to the ***eriss*** ecotoxicology laboratory.

Environmental consulting firms to target would include the existing commercial ecotoxicology laboratories in Australia (see next section for discussion of these) and key national/global and local companies. Large national/global companies include URS (www.ap.urscorp.com.au), SKM (see below) and GHD (www.ghd.com.au), while smaller firms include Hydrobiology Pty Ltd (Qld; www.hydrobiology.com.au), NIWA Australia (Qld; www.niwa.com.au) and HLA Envirosciences (www.hla-enviro.com.au).

Table 7 Potential industry clients in the Northern Territory for the ecotoxicology laboratory

Industry	Details	Source (if relevant)
Mining	*6 operating mines (3 coastal, 3 inland):* Alcan Gove, GEMCO, McArthur River Mining (Xstrata), Renison Consolidated Mines (Tom's Gully), AngloGold Australia (Union Reefs), Northern Cement	DBIRD (2003a)
	4 mine proposals (all inland): Batchelor Magnesium Project, Savanna Mineral Resources Pty Ltd; Browns Polymetallic Project, Compass Resources NL; Burnside Joint Venture, Burnside Operations Pty Ltd; Maud Creek Gold Project, Harmony Gold Operations Ltd.	DBIRD (2003b)
	8–10 closed mines (all inland): Annie Mine, Softwood Plantations Pty Ltd; Brock's Creek Mine, Burnside Operations Pty Ltd; Cosmo Howley Mine, Burnside Operations Pty Ltd; Merlin Mine, Ashton Mining Ltd; Rustler's Roost Mine, Rustler's Roost Mining Pty Ltd; Sandy Flat Mine, Redbank Copper Pty Ltd; Woodcutters Mine, Newmont Woodcutters Pty Ltd; Yimuyn Manjerr Mine, General Gold Operations Pty Ltd.	DBIRD (2003c)
Water	Power and Water Authority	
Oil and gas	Philips Petroleum Company Australia Pty Ltd / ConocoPhillips Woodside Petroleum Pty Ltd Santos (NGA) Pty Ltd Santos Offshore Pty Ltd Origin Energy Bonaparte Pty Ltd	
Horticulture & agriculture	Agrochemical companies including: Monsanto, Nufarm, Dow AgroSciences	
Aquaculture	Nutreco/Marine Harvest	

Competition

The major commercial ecotoxicology laboratories in Australia are:

- Sinclair Knight Merz (SKM) ecotoxicology laboratory (www.skmconsulting.com/environmental/science/index.cfm)[2];
- CSIRO Centre for Advanced Analytical Chemistry (www.det.csiro.au/science/researchservices/toxicity_topics.htm); and
- Ecotox Services Australasia (ESA; www.ecotox.com.au).

In terms of tropical freshwater ecotoxicology, there currently exists little competition from these groups. As mentioned above, CSIRO's Centre for Advanced Analytical Chemistry has a

[2] In mid-2004, Sinclair Knight Merz announced the closure of its ecotoxicology laboratory, leaving the two remaining listed facilities as the only major commercial aquatic ecotoxicology providers in Australia.

tropical algal species that it uses for commercial work, however, in the past, this group has usually passed on the additional tropical freshwater toxicity testing requirements to ***eriss*** (see table 5; it is uncertain if this has always occurred).

Major competition exists between the commercial laboratories for commercial marine ecotoxicology, both temperate and tropical. CSIRO has a stranglehold on the mining industry whilst ESA has a strong relationship with the oil and gas industry in the tropics. If ***eriss*** were to enter into the tropical marine ecotoxicology market it would seem most appropriate to provide complementary services to those already being offered. That is, the initial establishment of a marine ecotoxicology capability should focus on demand for additional testing capabilities using species not currently offered. Development of complementary capabilities enables the formation of alliances/agreements with other commercial ecotoxicology laboratories, an issue that is further discussed below, in *A proposed strategy*. ***eriss***'s location in Darwin may be seen by prospective clients in the tropics as a major advantage, perhaps sufficient to warrant the development of competing capabilities at some point in time. These types of issues and associated decisions need to be considered in conjunction with the scope and extent of any proposed marine ecotoxicology research capability – ie the two areas are highly inter-related.

Several other groups, including university, state government research institutions and small consulting firms undertake some commercial ecotoxicology, including:

- University of Technology Sydney (UTS) – fish toxicity testing only, including the tropical barramundi, *Lates calcarifer*;
- Griffith University – limited tropical commercial work usually using the shrimp *Caradina* sp., although commercial activities have probably ceased;
- Marine and Freshwater Resources Institute (MAFRI) – temperate marine ecotoxicology only;
- Leeder Consulting – mostly Microtox® testing, but some temperate freshwater invertebrate testing (eg shrimp, *Paratya australiensis*);
- Ecotox Pty Ltd – terrestrial ecotoxicology only;
- NIWA Australia – temperate ecotoxicology through NIWA in New Zealand.

It is unlikely that these groups will represent major competitors of ***eriss***. If a commercial tropical marine fish toxicity test is developed, then there might be some competition with the UTS barramundi test.

The above discussion does not account for the possible future expansion of existing laboratories or establishment of new laboratories in Australia (eg Golder Associates, through a subsidiary called BioQual, are establishing commercial laboratories for terrestrial ecotoxicology in Melbourne and possibly Brisbane). However, ***eriss*** will most likely be well-placed if it is able to capture and maximise the tropical freshwater market within the next 2 years.

Specific opportunities

At this point in time there exist several prospects for commercial ecotoxicology work that should be further pursued. These are briefly described below.

Release of $MgSO_4$-rich pit water from Argyle Diamond Mine

Argyle Diamond Mine discharges pit water rich in $MgSO_4$ into a local creek. Concerns regarding this, and discussions between various parties including Argyle Diamond Mine (Barry Muir, Environment Manager), ***eriss*** (Chris Humphrey), the WA Department of Environment Protection (DEP; Briony Lalor) and the WA Water and Rivers Commission (Susan Worley) have led to the apparent inclusion in the mine's licence agreement that the company assesses the toxicity of the waste water according to the recommendations of ANZECC and ARMCANZ (2000). As ***eriss*** is the only laboratory equipped to undertake tropical freshwater ecotoxicology, it is unlikely that the work would go elsewhere.

This represents a major opportunity to undertake a commercial project from WA and establish a strong relationship with the relevant government departments. Given that the timeline of this prospect is currently unclear, it is imperative that further contact be made with relevant WA government personnel (ie as listed above).

Seepage of Shoal Bay Landfill leachate

At a recent meeting between ***eriss*** (Rick van Dam and Chris Humphrey) and DIPE (Michael Lawton), the issue of leachate from Shoal Bay landfill site (owned by Darwin City Council (DCC) and operated on contract by Henry Walker) seeping into a nearby freshwater swamp was discussed. DIPE has previously discussed this issue with DCC and the possibility of some form of assessment of potential/actual impacts. It was made clear that ***eriss*** are keen, able and available to assist and have the full capability to undertake either or both an ecotoxicological assessment to determine toxicity (if any) of the leachate and a field survey (using monitoring techniques and relying on appropriate controls) to assess impacts (if any) of the leachate on the wetland.

Currently, ***eriss*** is awaiting further clarification and instruction from DIPE on a number of issues, including the possible development of a Brief and the form of a Tender process, if any. In the event that DIPE is not in regular contact, there needs to be regular (ie approximately fortnightly) follow-up in order to keep the issue active.

Other prospects

Several additional commercial prospects are listed below. Whilst no direct action is currently required towards further developing them, it would be worthwhile to maintain a 'watching brief' in order to be in a position to identify if and when they could/should be acted upon.

- *LNG facility – Pt Wickham*: This prospect has been sufficiently detailed above, in *Research Directions* (Directions and issues; marine ecotoxicology)
- *Assessment of retention pond water toxicity at Mt Todd Gold Mine:* Although some limited work on this issue was undertaken in 2002, there exists opportunity to undertake a more detailed assessment as a part of a larger consultancy proposal currently being negotiated with the Jawoyn Association to undertake a comprehensive assessment of the environmental status of the abandoned Mt Todd Gold Mine. This issue needs to be kept active with Dr Ken Evans, the ***eriss*** project manager for this proposal.

Quality Assurance and Quality Control

Quality in all aspects of a commercial project is critical in undertaking credible, defensible and financially viable work, and building and maintaining a sound reputation in the area of commercial ecotoxicology. This section outlines some key Quality Assurance (QA) and Quality Control (QC) requirements for undertaking commercial ecotoxicology.

Project management

Formal project management procedures should be in place and include:

- Standard quotation and proposal formats/templates;
- Clear and agreed (between the client and contractor) project scope, objectives, methodology, timeline, budget and reporting requirements;
- Clear and agreed roles and responsibilities of project team members and lines of reporting, including a Project Director and Project Manager/Coordinator;
- Effective risk identification and management procedures;
- Appropriate allocation, use and management of the required resources;
- Agreed communication plan between the client and contractor; and
- Regular/ongoing review of project progress and final review of project outputs and project success against specified criteria, by Project Director (or equivalent).

Formal project management training can provide the necessary knowledge and skills for effective project management. Initially, a check-list of the necessary major tasks required to successfully complete a commercial ecotoxicology project, from proposal to close-out stage, would contribute greatly to ensuring sound project management. Within the required project management tasks/skills, there should be an ability to accurately cost commercial projects and monitor project costs. It should also be noted that ***eriss***'s standard project approvals system already covers or has the scope to cover a number of the above procedures (eg risk management, use of resources, communication).

Laboratory and testing

QA in the laboratory includes the following:

- Existence of and adherence to fully developed and documented Standard Operating Procedures (SOPs) for all analytical tasks;
- Existence of and adherence to a fully documented Laboratory Procedures Manual for all other laboratory tasks including instrument calibration and maintenance, sample handling, equipment cleaning and test species culturing/husbandary; and
- Formal sample tracking and identification procedures.

QC in laboratory toxicity testing includes:

- Use of appropriate controls;
- Use of formal test acceptability criteria;
- Evaluation of test species sensitivity using reference toxicants; and
- Use of appropriate blanks and duplicates if samples are being analysed for chemical constituents.

A client's perception of and confidence in the provision of a quality service can be further assured if the laboratory has a recognised accreditation. The National Association of Testing Authorities (NATA) offers accreditation against the internationally recognised standard ISO/IEC 17025 (1999) *General requirements for the competence of testing and calibration laboratories*. However, the staff time required to gain and maintain NATA accreditation is probably prohibitive for a laboratory that primarily undertakes research activities. Nevertheless, the ***eriss*** ecotoxicology laboratory was NATA accredited in the early 1990s,

and the necessary laboratory documentation and procedures (ie SOPs and Laboratory Manual) are still generally in place. In addition, the Laboratory Manual is currently in the process of being updated to reflect recent procedural changes/refinements, although time limitations are preventing the staff from completing this at a sufficient rate. This means that in its current status, the ecotoxicology laboratory has in place the majority of necessary QA/QC procedures, perhaps with the exception of a regular reference toxicant testing program and adequate sample tracking procedures. At some stage in the near future, these procedures will need to be documented and implemented. An example sample tracking, or chain-of-custody form (from EVS Environment Consultants, Vancouver, Canada), for recording the movements of samples from the time of collection to receipt by the ecotoxicology laboratory, is provided at Appendix G. The issue of NATA accreditation may need to be reconsidered in the future if the laboratory shifts its focus from research to commercial activities.

Another quality certification that could be considered, but on an Institute or Division wide basis, is the broader ISO 9001:2000 *Quality management systems – Requirements*. ISO 9001 is the requirement standard used to assess a company's ability to meet customer and applicable regulatory requirements, and thereby address customer satisfaction. Although not specifically addressing laboratory quality issues, ISO 9001 does provide assurance to clients of an acceptable level of quality in the management of projects and systems. With the anticipated increase in the amount of commercial projects undertaken across SSD, such a quality accreditation may be worthwhile, although detailed consideration of this is not within the scope of this review.

Staffing

Staff need to be appropriately qualified and trained/experienced in the necessary tasks. For example, unless agreed by the client, it is generally unacceptable to have students work on commercial projects because they are often not sufficiently familiar with all the necessary technical aspects and associated QA/QC issues and tasks.

Sufficient staff need to be available to be able to undertake the work in the required time. The majority of commercial projects will require at least one professional level and two technical level staff members, with the former usually undertaking the project management, review and QA role and the latter the technical and QC role. Existing staff resources within the ecotoxicology laboratory are further discussed below, under *Constraints* and again under *Staffing Issues*.

Data/information

QA for data/information management includes:

- Independent check/review of data; and
- Effective hard and electronic data and records management systems.

It should be noted that the ecotoxicology laboratory's hard copy management system for toxicity test data is one of the most comprehensive and effective record keeping systems for ecotoxicology laboratories in Australia, both research and commercial. However, management of toxicity test data in electronic format is less impressive, with the major statistical software package being used, ToxCalc, not being fully set up according to the specific toxicity tests the laboratory undertakes.[3] This needs to be rectified. Finally, general

[3] All raw test data and statistical analyses are saved as MS Excel files under the appropriate project in SSD Explorer (the electronic information management system for Supervising Scientist Division).

project management information is appropriately managed in both hard and electronic form in accordance with Department of the Environment and Heritage guidelines.

Reporting

Quality reporting is essential in ensuring client satisfaction. Standard reporting templates assist in providing quality reporting. It may be desirable to have two standard templates: a 1–2 page summary report and a detailed report template. The decision on which reporting format is used needs to be pre-agreed with the client.

Key aspects of a detailed report include:

- Concise backgound/introduction of the issue being addressed including the context;
- Project aims and the hypotheses being tested;
- Methodology including sample transport and holding details, species tested and test details, QA/QC procedures and criteria, and statistical analyses;
- Clear and concise results of toxicity tests, chemistry (if relevant) and QC criteria;
- Discussion of the results in the context of the issue being addressed, including conclusions and recommendations, referring back to the project aims;
- Reference list;
- Appendices of additional relevant information (eg toxicity test raw data and statistical analysis print-outs).

As a final means of QA, and if agreed by the client, independent peer-review of final reports should be considered for larger projects.

Recommendations

27. That a check-list be developed, listing all essential major tasks for commercial projects, from proposal through to reporting and close-out stage.
28. That efforts be undertaken with ***eriss***/SSD to develop the capacity to accurately cost commercial projects and monitor and review project costs.
29. That a reference toxicity testing program be implemented for all routinely used species. This should probably be implemented in a tiered manner, with an aim to have established reference toxicant Control Charts for each species within 2 years.
30. That formal sample tracking procedures be developed and implemented for commercial projects.

Constraints

Research activities

Existing uranium mining research activities over the next 2 years will limit the number of commercial projects that can be undertaken. At this stage this is not considered a major constraint because the commercial business will still be establishing itself over this time and it is probable that the number of commercial projects will be manageable.

Future, non-uranium mining ecotoxicology research activities may also compete with commercial projects for ecotoxicology laboratory resources. Assuming that the ecotoxicology laboratory will be involved in externally funded research projects, there will be a need for careful and pro-active management of the laboratory's work program.

Staff resources

As at the time of commencement of this review (ie September 2003), the existing staff of the ***eriss*** ecotoxicology laboratory (see *Staffing Issues* below for details) did not have the relevant high level scientific, commercial and project management experience to appropriately undertake commercial ecotoxicology projects. Officially, the Ecotoxicology staff resources consisted of a laboratory technician (EA3 level) and an Ecotoxicology Research/Technical Officer (EA4 level), with two additional ***eriss*** staff members (an EA4 and EA5 level) experienced in some or all of the relevant ecotoxicology procedures. The existing technical/laboratory expertise is generally appropriate for undertaking commercial testing, although additional training or familiarisation of relevant QA/QC procedures and issues would probably be required. More importantly, there is a requirement for a senior ecotoxicologist with experience in the commercial ecotoxicology sector to be appointed to develop commercial opportunities, design, oversee and manage commercial projects, and be the contact point for clients. This is further discussed below, in *Staffing*.

Currently, with only two technical/laboratory staff, it would probably be possible to undertake only one commercial project at any one time. However, appropriate program and time management should minimise the likelihood of more than one commercial project having to be undertaken simultaneously.

Quality and reputation

The ecotoxicology laboratory does not currently have a reputation in the area of commercial ecotoxicology, although it has undertaken limited commercial work in the past. Consequently, it is imperative that a Quality System incorporating the types of aspects described above is implemented for all commercial projects, such that a good reputation is gained and maintained from the outset. To illustrate the importance of this, the poorly managed (semi)commercial project described above in *Track Record* did seem to damage to some extent the ecotoxicology laboratory's reputation with the relevant NT Government personnel, and it is likely that additional efforts will be required at convincing these personnel of the benefits of utilising ecotoxicology. The inability to gain a positive reputation or any lowering of an established reputation will almost certainly result in a reduction in the amount of commercial work the ecotoxicology laboratory receives.

A proposed strategy

As a preface to this section, it should be considered preferable that research, rather than commercial business, be seen as the primary function of the ***eriss*** ecotoxicology laboratory. That is, the generation of information, knowledge and understanding for the broader community on the risks and impacts of contaminants in tropical aquatic ecosystems should be the major goal of the ***eriss*** Ecotoxicology Program. The majority of this is achieved through research, however, a component would include helping Australian industries to improve their environmental performance, and is where ***eriss***'s ability to undertake commercial ecotoxicology should be utilised. However, it should be emphasised that the success of this strategy requires that the ecotoxicology laboratory be successful in securing large scale external research funds to support its non-uranium mining research activities.

Internal requirements

For the ***eriss*** ecotoxicology laboratory to have the ability to consistently undertake and complete commercial projects to a high standard (including on time and budget), there are a number of specific internal requirements that first need to be in place. Some of these are noted in the above sections with the key requirements specified in Recommendations 27 to 30. These requirements/tasks should be addressed as early as possible within the constraints of the current research work plan, with every attempt made to have them completed prior to securing a commercial contract.

Business development/marketing requirements

Business development for the ecotoxicology laboratory's commercial services could be focused on the following strategies:

1. Regularly liaising with key local and national environmental consultancies, including other commercial ecotoxicology laboratories, to ensure they are fully aware of the ***eriss*** ecotoxicology laboratory's capabilities and willingness to collaborate on commercial projects;
2. Direct and regular liaison with relevant government agencies; and
3. Direct and regular liaison with targeted industries/prospective clients.

Promotional material for the ecotoxicology laboratory's facilities and capabilities has recently been produced in the form of a professionally prepared double-sided, A4 size brochure and updated information on the Ecotoxicology pages of the SSD web site, which includes access to an electronic copy of the brochure:
(www.deh.gov.au/ssd/wetlands/assessment/ecotoxicology.html).

Contacts within the identified consultancies, government agencies and prospective clients should be notified of the existence of the ***eriss*** ecotoxicology laboratory and its capabilities, and sent copies of the promotional material and directed to the web site information. Follow-up phone calls or E-mails (preferably the former) are useful for ensuring the material has been received/read and provides the opportunity to answer any questions or provide points of clarification.

In the first instance, the majority of effort should be directed towards the development of positive relationships with the key consulting firms, as this will likely provide the greatest return per unit effort. Where the firms have a local (Darwin) presence, it may be beneficial to invite the key contacts to ***eriss*** to inspect the Laboratory and discuss potential opportunities/collaborations.

Communication with relevant government agencies should encourage the incorporation of ecotoxicological assessment in discharge licence requirements, chemical registration requirements, etc. This represents an investment towards longer-term benefits to the Laboratory as well as providing the possibility of being alerted to upcoming issues or specific prospects.

Recommendation

31. That a short but formal marketing and business development plan be produced based on the information in this section, which details relevant activities, timelines and monitoring/evaluation procedures.

Laboratory issues

A component of this review was to consider and provide advice on a number of laboratory related issues that were paramount in the future success of the ecotoxicology laboratory, both from a research and commercial perspective. Each of these issues are discussed below.

Metal contamination in toxicity tests

Since the ***eriss*** laboratories relocated to Darwin, the ecotoxicology laboratory has regularly detected contamination of several metals, namely copper (Cu), zinc (Zn) and to a lesser extent, aluminium (Al) in toxicity test solutions. Discussions with staff and inspection of chemistry data sheets indicated that the contamination was usually independent of the treatment concentrations or dilutions.

Staff had already undertaken some investigations to identify the source of the contamination. In these investigations, specific procedures were targeted (eg filtering of dilution water, autoclaving of test media, contamination from laboratory gloves during handling). Copper concentrations measured in samples handled or treated in different ways are presented in table 8. Although the results did not identify conclusively any particular source of contamination, chemical analyses of unfiltered Darwin tap water (DTW) revealed extremely high concentrations of several metals, particularly Cu (table 8) and Zn. Prior to the investigations into the contamination source, DTW was the water source used for the initial wash cycle in the ecotoxicology laboratory's analytical dishwasher, and was also used for general laboratory bench cleaning and hand washing. Filtered Darwin tap water (FDTW), which is DTW that has been passed through a gravel and activated carbon filter, and is the water source for the laboratory's fish, hydra and cladoceran cultures (although hydra and cladoceran cultures are also maintained in Magela Creek water), also contained considerable (but much lower than DTW) concentrations of Cu (table 8) and to a lesser extent Zn.

From the investigations undertaken to date, the most likely source of contamination appears to be the DTW used in the first dishwasher wash cycle and for general benchtop cleaning and hand washing. An additional source of contamination during whole effluent toxicity testing may be the 10 μm filter papers used to filter the test diluent (when it is a natural receiving water) and test sample, although this has not yet been confirmed. Discussions with staff at ***eriss*** that have experience in working in chemistry and clean laboratories, namely Claudia Sauerland and Andreas Bollhoefer, identified several other potential sources of contamination, including the bleached 'Day-Lee' towels used for wiping benchtops and drying filtration equipment (the bleaching pigment contains high levels of Zn), contact of test solutions with corroding springs in pipetters, and poorly managed acid washing/soaking procedures.

Since discovering the high concentrations of some metals in DTW, and identifying other potential sources of contamination, the following actions have been implemented:

- Re-plumbing the laboratory dishwasher such that it uses only Elix (de-ionised) water for all washing and rinsing phases;
- Re-washing all laboratory glassware in the re-plumbed dishwasher;
- Cleaning/wiping benchtops and laboratory equipment only with Milli-Q water;
- Rinsing/washing hands only with Milli-Q water;
- No longer using bleached hand towels to wipe benches and dry laboratory equipment;

Table 8 Copper concentrations measured in different samples handled or treated in different ways

Water Type	Treatment	Date	Cu (μg/L)
DTW	None (samples taken from tap in lab, not pre-filter)	14/05/03	**92**
		11/09/03	**1040**
DTW	None (samples taken from newly-installed tap prior to DTW entering activated carbon filters)	11/11/03	**48**
		09/01//04	**19**
FDTW	None	08/08/02	0.16
		26/08/02	2.2
		19/11/02	**11**
		17/12/02	**134**
		07/04/03	**8.5**
		14/05/03	**5.9**
		02/06/03	**6.0**
		08/07/03	**7.4**
		24/07/03	**4.4**
		13/08/03	**5.4**
		11/09/03	**4.6**
		11/11/03*	2.8
		09/01/04*	2.8
MCW	Filtered through #42 (10 μm) Whatman filter paper	04/06/03	0.38
MCW	Not filtered through #42 (10 μm) Whatman filter paper	04/06/03	0.28
Milli-Q	None (sample taken directly from Milli-Q outlet into sample bottle)	08/08/02	0.16
		26/08/02	0.09
		18/12/02	**0.93**
		02/06/03	0.31
		24/07/03	0.09
		13/08/03	**0.76**
		11/09/03	0.09
		??/10/03	**1.5**
		31/10/03	0.2
		11/11/03	0.45
		12/11/03	**0.76**
		09/01/04	<0.01
Milli-Q	Filtered through #42 (10 μm) Whatman filter paper	07/04/03	**6.3**
		04/06/03	0.57
		18/10/03	0.20
		12/11/03	**1.9**
		09/01/04	0.09
Milli-Q	Sampled from beaker before filtering through #42 (10 μm) Whatman filter paper	07/04/03	0.11
		04/06/03	0.71
		18/10/03	0.07
Elix	None (sample taken directly from storage tank into sample bottle)	Not specified	0.04

* denotes measurements of samples collected after replacement of DTW filter media (sand/gravel + activated carbon)

- Checking/servicing of all pipetters;
- Ensuring regular renewal of nitric acid baths used for acid soaking of glassware;
- Replacing the filter medium in the flow-through water filter system with a higher (hospital) grade of activated carbon for increased removal of metals and chlorine.

Summarised details of the investigations, information gained and actions taken are provided at Appendix H.

Since the above actions were taken, chemical analyses from laboratory toxicity tests have revealed much less but still occasional contamination of copper, zinc or aluminium. For example, for the Ranger uranium mine pre-release toxicity testing in December 2003, which involved a total of three toxicity tests (ie one toxicity test each for the three species, *M. macleayi*, *H. viridissima*, *M. mogurnda*), only two out of 20 samples contained copper at greater than 1 μg/L (1.13 and 1.62 μg/L cf. general background of <0.5 μg/L), whilst one of of 20 samples experienced zinc contamination (12.9 μg/L cf. background of ≤3 μg/L). It should be noted that none of these levels of contamination represent a toxicological concern to the test species. At this stage, the frequency and magnitude of metal contamination in test samples appear acceptable, providing the contamination is closely monitored and further discussions are held with relevant experts to try to further reduce the contamination incidence. Efforts to further identify contamination sources are already underway, with duplicate samples of test solutions being taken to determine whether contamination is occurring during the preparation of the test treatment/dilution/concentration or during the collection of the test solution sub-sample for chemical analysis.

Recommendation

32. That the frequency and magnitude of Cu, Zn and Al contamination in test samples and the concentrations of these metals in DTW and FDTW are closely monitored, and that if necessary, further discussions are held with relevant experts and/or further investigations are undertaken to try to further reduce the contamination incidence.

Flow-through water delivery system

Since its relocation from Jabiru to Darwin, the ecotoxicology laboratory has experienced numerous and ongoing difficulties with the flow-through water delivery system used for the culturing of purple-spotted gudgeon (and other cultures, eg lemna, snails) in the indoor wet laboratory. For clarification purposes, the water delivery system can be considered to consist of three sub-systems:

1. *Filtration system:* DTW is pumped through two automated filters containing gravel and activated carbon, designed to remove calcium and metals in one pass;
2. *Header tank and heating/chilling system:* filtered DTW (FDTW) is fed under mains pressure to two 500 L recirculating header tanks with a single heater/chiller element; and
3. *In-lab plumbing and aquaria:* FDTW is gravity fed via a restricting valve flow meter to allow a maximum flow of 500 L h^{-1} FTDW to the aquaria in the wet laboratory, with the outflows from the aquaria directed via drains to the sewer.

Before discussing the major problems, the following points provide important contextual information on the issue:

- The previous fish culturing facility in Jabiru involved a static-renewal system, not flow-through system. Thus, a flow-through system was a new concept to most staff.
- Due to the resignation of a key staff member during the laboratory design phase, there was a discontinuity of staff overseeing the design and construction of the flow-through system.
- The original specification for the system required the ability to deliver FDTW to the wet laboratory at a minimum flow rate of 450 L h^{-1} at a constant temperature of 27±1°C. This was based on a flow rate to each aquarium of 15 L h^{-1} for a total of 30 aquaria with a total volume of ~3500 L (consisting of 6 × 288 L and 24 × 72 L aquaria). The hourly flow rate estimate was loosely based on achieving 99% molecular turnover over a 24 h period for the smaller (72 L) aquaria, calculated from Sprague (1973). This criterion was chosen as it would be a requirement in the event that the laboratory's 72 L aquaria might be used in the future for flow-through toxicity testing purposes.
- The hydraulic engineers that designed the filtration and header tank and heating/chilling system had no previous experience in designing such systems, although they assured ***eriss*** that it would meet the requested specification.
- An aquarium and water system expert from the Territory Wildlife Park, Dave Wilson (now a private aquaculturist), was contracted to provide advice on the water delivery system and construct all the associated plumbing and aquarium facilities in the wet laboratory.
- As a consequence of the discontinuity of information regarding the specifications of the water delivery system and final wet laboratory fit-out, the final aquarium composition was different from that originally proposed (and described above), instead consisting of 7 × 288 L and 21 × 144 L aquaria with a total volume of 5040 L, approximately 1500 L greater than the original specification.

The major problems encountered with the water delivery water system were that when staff increased the flow rate to the aquaria, the temperature would not hold at the specified 27±1°C and the header tanks would run dry. However, the laboratory staff had not yet quantified the total flow rate to the aquaria, so it could not be determined whether or not the system was operating at its intended specifications. Consequently, the staff were requested to calculate the existing flow rate to the aquaria, then sequentially increase the flow rate until the system failed, hence gaining an understanding of its capacity.

The flow rate data were recorded in the *Aquaculture Water System Maintenance Log Book* and are summarised as follows. Total flow rate of the system as it had been running for some time, which comprised 16 of the 28 aquaria receiving water, was 294 L h^{-1}, with an average flow rate per aquarium of around 18 L h^{-1}. There was little variability in flow over the course of the day, with 2-hourly measurements over the following 6 hours giving flow rates of 300, 294 and 294 L h^{-1}. When increased to a total flow rate of around 600 L h^{-1}, at an average per aquarium of around 37 L h^{-1}, both the header tank water level and incoming water temperature were still maintained. However, when flow rate was increased to 1300 L h^{-1}, the header tank water level could not be maintained. Thus, the results suggest that the water delivery system is indeed operating to, and even above, the specifications it was designed to meet.

However, the maxium total volume of 5040 L for the existing aquaria is substantially greater than the volume of ~3500 L on which the initial minimum flow rate of 450 L h^{-1} was calculated. Based on a minimum 99% molecular turnover over 24 h for the smaller 144 L aquaria (and using this flow rate also for the 288 L aquaria), a minimum total flow rate of 840 L h^{-1} would be required in the event all aquaria were receiving water. It is unclear at this stage whether the water delivery system would cope with such a flow rate. Nevertheless, it is considered that a flow rate of around 600 L h^{-1} will be more than sufficient to maintain adequate water quality in the aquaria, because:

- it is unlikely, at least in the near future, that all 28 aquaria will be receiving water; and
- the criterion of 99% molecular turnover of water over 24 h is based on a toxicity testing, not culturing, requirement and it is highly unlikely that the aquaria will be used for toxicity testing.

Discussions with laboratory staff and Mr Scott Smith, the plumber contracted to maintain the flow-through system, identified several additional problems with the flow-through system. These included the coating of the inner surfaces of the PVC piping and header tanks with a thick layer of red-brown material and problems with the size and fit of the ball float valve controlling flow rate from the filters into the first header tank. A list of solutions to the above problems was supplied by Scott Smith, and included:

- Installation of a pair of 5 μm pre-filters (such as those servicing the Milli-Q systems), prior to the activated carbon filters, to remove larger particles from the mains water prior to entering the filters and reduce the rate of build up in the post-filter piping and header tanks;
- Replacement of the existing ball float valve at the first header tank inlet with a larger valve in order to maximise flow rate from the filters into the tank; and
- Replacement of the existing ball-float arm with a shorter arm so that it does not need to be bent downwards, which is currently reducing the maximum volume of the first header tank.

As the water delivery system represents a new piece of equipment to the ecotoxicology laboratory, Mr Dave Wilson has been contracted by ***eriss*** to produce an SOP covering the details of the water delivery system, including its specifications, design, maintenance requirements and limitations. This document, expected for completion in early 2004, will consolidate the information on and requirements of the water delivery system and will make a significant contribution to minimising major technical problems.

Given the above improvements to the water delivery system and the results of the flow rate trials, and noting that there is ongoing monitoring of the system's performance, further actions or recommendations are considered unnecessary.

Stock culturing

At the time of commencement of this review, the ecotoxicology laboratory staff were experiencing several problems with the culturing of a number of the test species, namely *M. macleayi* and *H. viridissima*. The problems generally concerned poor culture health and regular culture crashes and there were suspicions that the problems may have been related to the use of FDTW as culture water following the relocation to Darwin.

Separate *M. macleayi* cultures kept in FDTW and MCW experienced unacceptable mortality rates and regular culture crashes, suggesting a stress unrelated to the culture medium. Nevertheless, several modifications were made to the *M. macleayi* culture media, involving the addition of different proportions of Perrier™ mineral water, often used as a minerals

supplement for culturing other cladoceran species, in both FDTW and MCW (ie 5% and 10% Perrier in FTDW and MCW). Survival and reproduction were monitored over a 5 week period, with no apparent differences in these parameters between culture media. The investigations that were undertaken all indicated that the cause of the problem was common to both the FDTW and MCW cultures, and was most likely a nutritional issue. International cladoceran experts Professor Donald Baird (Canada) and Dr Carlos Barata (Spain), both of whom have had previous associations with the ***eriss*** ecotoxicology laboratory, suggested, amongst other things, that a second algal species be used to supplement the cladoceran's food source. In November 2003, a culture of *Selenastrum capricornutum* was obtained from CSIRO for this purpose, but it was about this point in time that one of the laboratory staff noted that the stock solution of Vitamin B_{12}, which is added to the FFV cladoceran food mixture and acts as a nutritional supplement, was 1000× higher than it should have been. Further, the preparation date of the stock solution roughly corresponded to the time at which the *M. macleayi* cultures began to experience health problems. A new Vitamin B_{12} stock and FFV batch was immediately prepared and incorporated into the feeding regime, with rapid beneficial results on the health of the cladoceran cultures. Within several generations, MCW stock culture adult mortality was within acceptable limits and there have been no culture crashes since. In addition, a valid *M. macleayi* toxicity test (in MCW) was completed in December 2003, something that had been almost unachievable for the previous 12 to 18 months. However, FDTW cultures, although more healthy than previously, have still not been meeting acceptable survival levels. Thus, whilst a key cause of the *M. macleayi* culture health problem has been identified and overcome, there are still outstanding issues for the FDTW cultures that require further consideration and investigation. Although the Vitamin B_{12} stock problem appears to have been overcome, the importance of double-checking calculations prior to preparing culture medium stock solutions cannot be over-emphasised. Such checking should be implemented as part of the ecotoxicology laboratory's QA system.

Unlike the *M. macleayi* cultures, at the commencement of this review, *H. viridissima* bowl cultures kept in MCW were consistently healthier than the FDTW bowl cultures and had been so for several weeks, suggesting the FDTW may have been having an adverse effect. Hydra numbers were lower and the incidence of 'clubbing' (a response to stress) was greater in the FDTW bowl cultures. However, the back-up cultures, kept in an aquarium housing an archer fish and receiving FDTW under flow-through conditions, had been consistently healthy. It was thought possible that the 'conditioning' of the FDTW by the archer fish was improving the conditions for the hydra, possibly by increasing the amount of available nutrients in the water column. Consequently, it was recommended that another bowl culture be established using 'conditioned' FDTW from one of the aquaria housing fish, with the health of the culture monitored against that of the MCW and FDTW bowl cultures. Soon after this culture was established (ie mid September 2003), and for no reason that was obvious to the laboratory staff, the health of the FDTW bowl culture improved to a point where it was similarly or only slightly less healthy than the MCW culture. After approximately 4 weeks (ie mid October 2003), the 'conditioned' FDTW bowl culture, which had remained healthy for the duration, was no longer required and was discarded. At the time of completion of this review, the FDTW *H. viridissima* bowl cultures had consistently been in good condition for around four months. The cause(s) of the FDTW bowl culture ill-health, which had occurred for around a 6 week period, is still not evident. One potential cause is a change in water quality due to a change in the source of the Darwin mains water, however, this was not evident from measurement of water parameters such as pH and conductivity.While procedures to minimise culture crashes and ill-health are essential, it needs to be recognised that such problems are not entirely unavoidable. Whilst of some concern, this specific problem with *H. viridissima*

demonstrated that the ecotoxicology laboratory's 'back-up' systems and ability to respond to the problems were in place and effective. Consequently, there is no need for any recommendations regarding this issue.

Purple-spotted gudgeon (*M. mogurnda*) stock are housed in flow-through aquaria in the wet laboratory, with the majority used as breeding stock. Historically, at ***eriss***, it has been difficult to maintain productive and consistent *M. mogurnda* breeding stock. However, since the relocation to Darwin, this has proved an even more difficult task. Efforts during August/September 2003 to consolidate and expand the *M. mogurnda* breeding stock were, on appearance, proving successful. However, inspection of the fish tank log books indicated that of the 7 well-established breeding stock aquaria, only 2 groups had been producing an acceptably consistent supply of eggs, with another group producing batches of eggs intermittently. Additionally, many of the fish appear in poor condition, at least externally (eg damaged fins and tails) compared to stock maintained at the Jabiru Field Station (A Hogan, ***eriss***, pers comm). In mid-December 2003, the female in the most productive aquarium died. Consequently, there was inadequate availability of good quality eggs/larvae for the 2003–04 Ranger pre-release toxicity testing program. The use of spawning cues, including rapid water temperature changes and providing live meal worms as a food source were used to stimulate fish spawning, but proved only partially successful. Further efforts at improving the reproductive performance of the breeding stock are being continued, including the continued provision of live food, the use of aquatic plants for fish refuge and the re-distribution of individuals between aquaria. However, it is clear that more serious and comprehensive thought needs to be given to this issue, and this should include further discussions with existing staff with relevant expertise and local fish aquaculturists (such as Mr Dave Wilson). Discussions should focus on approaches for improving *M. mogurnda* reproductive output, but should not exclude consideration of the possibility of alternative test species (eg rainbowfish). This is further discussed below, in *Refinement of Test Procedures*.

No major problems were being encountered with the remaining stock cultures in the ecotoxicology laboratory. Briefly, the *Chlorella* sp. cultures were consistent and generally healthy, although it was noted by the laboratory staff that the cultures were no longer axenic. It was recommended that at some point in the near future, the culture be treated for removal of the bacterial contamination. This could be undertaken either in-house, using appropriate instruction, or by sending a sample of the culture to CSIRO. The *L. aequinoctialis* cultures have been healthy and consistent with excellent growth and very few incidences of fungal/bacterial contamination. The system of back-up cultures for both *Chlorella* sp. and *L. aequinoctialis* are in place and appropriate.

Recommendations

33. That further consideration and investigation is given to the health problems experienced by *M. macleayi* cultures in FDTW.
34. That, as part of the ecotoxicology laboratory's quality system, a procedure be implemented to ensure that all mathematical calculations required for culturing and toxicity testing purposes are double-checked by another member of the laboratory staff.
35. That further discussions with relevant staff and external experts are held to consider and agree on approaches for ensuring a consistent supply of fish eggs/larvae when needed, for toxicity testing purposes.
36. That the *Chlorella* sp. culture be treated for removal of bacterial contamination.

Refinement of test procedures

The ecotoxicology laboratory has six established toxicity testing protocols (covering five species), as recently published by Riethmuller et al (2003), and at least one other partially developed protocol (freshwater snail, *Amerianna cumingi*, 7–8 d reproduction test – which is already used for creekside toxicity monitoring at Ranger). However, there exists scope for the improvement and refinement of several of these toxicity testing protocols, as described briefly below.

Chlorella sp. 72 h growth inhibition test

The existing algal test uses a test volume of 50 ml per treatment in 250 ml Erlenmeyer flasks. In recent years, commercial laboratories in Australia have scaled-down the test volumes to 15 ml per treatment (using glass scintillation vials as test vessels), resulting in efficiencies of scale including reduced test preparation times and space usage. It may be considered worthwhile to investigate the feasibility of scaling-down the *Chlorella* sp. test, including any effects on test reproducibility and test species sensitivity.

L. aequinoctialis 96 h growth inhibition test

In 1999, the ***eriss*** ecotoxicology laboratory purchased the Leica QWin Image Processing and Analysis System software for its digital microscope camera and microscope apparatus. The software has good potential as a tool for capturing and quantifying a range of response data from toxicity tests. In particular, it could be used for the *L. aequinoctialis* population growth test to automatically count variables such as frond number, frond area, percent cover and/or potentially more sensitive responses such as average frond size/area and necrosis. The benefits of using the software would include reduced test counting/data collection times and the ability to measure new and potentially more sensitive endpoints. During the 2003–04 budget bids process, it was recommended to the Ecological Risk Assessment Program Leader that the training budget include an allocation for the ecotoxicology laboratory staff to undergo the appropriate software training by Leica staff.

In late 2002/early 2003, the *L. aequinoctialis* test was broadened to enable the use of natural MCW as the test diluent. The work was undertaken by ecotoxicology laboratory staff and a Northern Territory University (NTU) summer scholarship student, Kate Wagner. In order to meet the minimum acceptability criterion for control growth, the MCW diluent requires the addition of NO_3 and PO_4 only as nutrient supplements. To determine the appropriate nutrient concentrations, information was used from a current PhD study being undertaken at ANSTO by Amanda Charles, who had found that the concentrations of NO_3 and PO_4 required for *L. aequinoctialis* in synthetic Hawkesbury Nepean Water (a synthetic soft water simulating the inorganic composition of Hawkesbury Nepean River water) were 0.1 and 0.01 mg/L, respectively (representing a NO_3:PO_4 ratio of 10:1). However, initial experiments with *L. aequinoctialis* in MCW resulted in very little growth at these nutrient levels, probably because MCW is far more minerally dilute than synthetic Hawkesbury Nepean Water. Following investigation of higher nutrient concentrations, 88 mg/L NO_3 and 18 mg/L PO_4 were chosen as the final nutrient concentrations for MCW. However, at these concentrations, NO_3 and particularly PO_4 have the ability to complex significantly with uranium, thereby reducing its bioavailability (Dr Scott Markich, ANSTO, pers comm). Further, the ecotoxicology laboratory staff could not explain why the NO_3:PO_4 ratio was now 5:1, not 10:1. Review of the experiments undertaken and their resultant data indicated that the investigation was not sufficiently systematic or thorough, due largely to time constraints. From the data, there was an indication that the addition to MCW of 10 mg/L NO_3 and 1 mg/L PO_4 was sufficient for acceptable control growth. Consequently, there is a need to re-

determine the minimum NO_3 and PO_4 concentrations required to be added to MCW in order to consistently exceed the minimum acceptability criterion for *L. aequinoctialis* control growth.

A. cumingi 7-d reproduction test

The *A. cumingi* 8 d reproduction test was adapted to the laboratory from the creekside monitoring protocol (Humphrey et al 1995) by Clint McCullough in the late 1990s, as part of his PhD project. More recently (late 2003/early 2004), the protocol was further adapted/refined to laboratory conditions by ecotoxicology laboratory staff and an NTU summer scholarship student, Anthony Navidad. In particular, the aim of the recent research was to reduce the high intra-treatment (control) variability in the number of snail embryos after 8 d, this being the major endpoint. For the experiments, mature snails, which had formerly only been cultured in outdoor tubs at the Jabiru Field station, were brought to Darwin and housed and cultured under controlled light (12 h light : 12 h dark photoperiod) and temperature (30°C) conditions in the ecotoxicology laboratory's indoor wet laboratory. Snail embryos/larvae from one large parental stock were isolated every 3–4 weeks for rearing as future test animals. In this manner, snails of similar size and a relatively similar age were used for testing purposes. In contrast, the creekside monitoring protocol and the approach used by Clint McCullough uses snails of similar size but unknown age. Briefly, the major findings of the project included:

- Test volume could be reduced from 5 L per replicate to 2.5 L per replicate;
- Daily water changes were sufficient to keep NH_3 below 1 mg/L;
- When similar size and age animals were tested under tightly controlled laboratory conditions, variability of control egg mass and embryo numbers was greatly reduced compared to Clint McCullough's research;
- Six pairs of snails are required per replicate in order to sufficiently minimise variability between replicates, and that 6 pairs of snails can be maintained in 2.5 L of water with daily water renewals; and
- Toxicity of $MgSO_4$ was similar over a 5 d or 7 d test duration.

This work provided important information towards the successful development of a laboratory toxicity test for *A. cumingi*. Most notably, the unacceptably high control variability measured in previous work was reduced to acceptable levels. However, it was not possible to determine whether this was due primarily to the use of snails of known, similar age as well as size or the tests being carried out under fully controlled laboratory conditions, or a combination of both. Thus, in order to optimise the efficiency as well as sensitivity and reproducibility of the *A. cumingi* protocol, differences in reproduction and associated reproductive variability between similarly sized snails of known, similar age and similarly sized snails of unknown age need to be determined. The outcomes will establish the minimum culturing requirements/effort for *A. cumingi*, which ultimately, will dictate the viability of using the snail as a regular test species.

M. mogurnda 96 h larval survival test

As described above, since relocating the facilities to Darwin, the ecotoxicology laboratory staff have experienced difficulties in establishing productive and consistent *M. mogurnda* breeding stock. However, this was always a challenge long before ***eriss*** relocated to Darwin, and relates to the fact that the *M. mogurnda* reproductive strategy is not an ideal one for toxicity testing purposes. Reproductively mature *M. mogurnda* breed in pairs. Coupled with the fact that they can only be kept at low densities due to their aggressive nature, this results

in a heavy reliance on individual animals. For example, 288 L breeding aquaria usually consist of one female and one to three males (Riethmuller et al 2003). If the female is not in reproductive condition, then that breeding stock is entirely non-productive. Contrast this to other types of fish species used for toxicity testing, such as rainbowfish (*Melanotaenia* spp.), which spawn in groups and can be kept at higher densities, and where a few non-reproductive individuals will have minimal effect on the productivity and consistency of the breeding stock. Thus, in addition to Recommendation 35, above, it may be worthwhile considering the use of an alternative, or at the least, additional fish species for toxicity testing. Candidate species include the black-striped rainbowfish (*Melanotaenia nigrans*), which is currently used as one of the creekside monitoring test species and for which a preliminary laboratory protocol has been developed (Williams et al 1998), or the chequered rainbowfish (*Melanotaenia splendida inornata*), which generally is more fecund than the black-striped rainbowfish and has previously been used at ***eriss*** (and ERA) for toxicity testing purposes (Holdway 1992). This represents an important but not urgent issue, and could be appropriately addressed through a student (eg Honours) project, thus, minimising staff resource requirements.

Recommendations

37. That the feasibility of reducing the per replicate test volume for the *Chlorella* sp. population growth test from 50 ml in a 250 ml Erlenneyer flask to 15 ml (in a glass scintillation vial) be investigated.
38. That the 2003–04 Ecological Risk Assessment training budget include an allocation for the ecotoxicology laboratory staff to undergo the appropriate image analysis software training by Leica staff.
39. That the minimum NO_3 and PO_4 concentrations required to be added to MCW in order to consistently exceed the minimum acceptability criterion for *L. aequinoctialis* control growth over 96 h be determined.
40. That differences in reproduction and associated reproductive variability between similarly sized snails (*A. cumingi*) of known, similar age and similarly sized snails of unknown age need to be determined, in order to establish the minimum culturing requirements/effort for the *A. cumingi* test.
41. That the existing *M. mogurnda* toxicity test be adapted for using a rainbowfish species, probably black-striped rainbowfish (*Melanotaenia nigrans*) or chequered rainbowfish (*Melanotaenia splendida inornata*). This could be completed as a student (eg Honours) project.

General laboratory procedures

General laboratory procedures are documented in the ***eriss*** *ecotoxicology laboratory Manual*. This document has existed and been periodically updated since the early 1990s, when the laboratory was accredited by NATA. The Laboratory Manual consists of four sections: *General laboratory maintenance*; *Invertebrate and plant maintenance*; *Fish maintenance*; and *Test protocols*. It is a very comprehensive document that covers all necessary aspects of an ecotoxicology research laboratory, and, from my experience, represents the majority of documentation required for a commercial laboratory. Since the relocation to Darwin, there

has been a need to revise a substantial portion of the Laboratory Manual as it was very specific to the Jabiru ecotoxicology laboratory. This revision has been occurring at a steady rate, as spare time from general laboratory maintenance and toxicity testing has allowed. In addition, there has been the need for additions to the Laboratory Manual, due to the purchase of new equipment and facilities (eg the flow-through water delivery system, dishwasher, fume cupboard, demineralised water system, etc.). Again, new procedures have been added as time has allowed. From discussions with staff it appears that procedures are still being followed as laid out in the Laboratory Manual and where changes to procedures have occurred these have been justified and the Manual amended accordingly. It should be emphasised that revisions to Laboratory Manuals are an expected and normal process, as existing procedures are refines/improved and new ones adopted. The key issue then, which appears to be addressed by the ecotoxicology laboratory staff, is that there is an awareness and mechanism for ensuring that procedural changes are formally documented.

Comprehensive information is collected and clearly recorded in a series of Log Books on the status/condition of laboratory cultures and equipment. Routine laboratory maintenance tasks are listed and clearly displayed in the Laboratory on weekly or annual maintenance charts (see Appendix I). Discussions with laboratory staff have indicated that these tasks are almost always undertaken as scheduled, although some slippage has occurred, particularly with the monthly checkpoint calibrations for equipment such as pipettes, balances and maximum-minimum thermometers. Actions have been implemented to ensure all outstanding tasks are completed. With the exception of the coulter counter, servicing of all major equipment (eg Milli-Q/Elix demineralised water system, microscopes, balances) is undertaken on an annual basis and is fully up to date. Maintenance and servicing of the Coulter products has changed to a new supplier, and ecotoxicology laboratory staff have been making attempts to find this new supplier.

Staffing

Existing resources

At the commencement of this review (ie August 2003), the existing staff resources of the ecotoxicology laboratory were:

- Alicia Hogan, Research Officer – EA4 level (acting at EA5 level; PN351); and
- Suthidha Nou, Laboratory Technician – EA3 level (PN352).

Alicia Hogan is an ongoing employee, while Suthidha Nou was on a temporary 6 month contract from July 2003 until early January 2004.

The laboratory staff are well qualified and trained and highly competent in their duties, although Suthida Nou is still undergoing training in a number of the toxicity testing protocols. They are supported by the Program Leader of the Ecological Risk Assessment Program, Dr Peter Bayliss (PRS), who does not have a background in ecotoxicology, and at least one additional staff member, James Boyden (EA5), who has some experience in ecotoxicology, and assists with general laboratory maintenance when necessary (ie when one or both of the permanent laboratory staff are unavailable). An Honours student, Kate Wagner, who has some experience in ecotoxicology also assists with general laboratory maintenance when necessary. There is an additional staff member, Caroline Camilleri (EA5), who has extensive ecotoxicology experience, but has not worked in the ecotoxicology laboratory since early 2003. As noted above, in *History*, the ecotoxicology laboratory has been without a senior

ecotoxicologist since September 2001, although a vacant appointment for an EA6 level ecotoxicologist does exist, but had not been filled.

Discussions with staff have indicated that the existing staffing level has not been sufficient to adequately maintain the ecotoxicology laboratory's equipment, supplies and test species cultures to the level of quality preferred. Consequently, only very limited project work/experimentation could be undertaken. A significant amount of the toxicity testing undertaken during 2002–03 was done with the aid of students (ie PhD student, Clint McCullough, and NTU summer scholarship students, Kate Wagner and Anthony Navidad). In addition, higher responsibility project development and management tasks, usually the responsibility of more senior positions (ie EA6 or above), often fell to the existing laboratory staff, further reducing time available for these staff to undertake experiments. In short, it is clear that the various constraints on the current activities, output and future of the Ecotoxicology program are due almost exclusively to the inadequate staffing allocation within the group.

Implications

At the commencement of this review, the two major staffing allocation issues relating to the ecotoxicology laboratory's ability to perform its functions were the lack of a senior ecotoxicologist and the possible non-continuation of the existing Laboratory Technician temporary contract (PN352) beyond January 2004. A summary of the implications of these staffing problems is provided in table 9.

Table 9 Summary of implications of insufficient level of staffing in Ecotoxicology Program

Lack of Senior Ecotoxicologist	Non-continuation of Technical Officer contract (PN352) beyond June 2004
Uranium mining Research	
Slowed progress on research due to EA-4 Officer having to manage administrative, liaison & report writing tasks	Little if any progress on research beyond June 2004
Insufficient expertise for data interpretation and subsequent impact on work to come	Difficulty in maintaining laboratory cultures
Insufficient expertise/resources for reporting and publication	
Non- uranium mining Research	
Limited ability to set and implement strategic direction of ecotoxicology program	Limited ability to expand capabilities of the ecotoxicology program to accommodate future research directions (eg marine ecotoxicology)
Limited ability to undertake commercial work due to lack of senior contact/project manager	Limited ability to undertake commercial work due to shortage of technical staff
Limited ability to communicate/liaise with Government and industry on relevant issues	Limited ability to undertake externally funded research due to shortage of technical staff
Limited ability to collaborate with other institutions and to apply for external research funding	
Reduced chances of gaining external research funding	
Limited ability to host postgraduate students due to lack of on-site supervisor	

At this existing staffing level, the ecotoxicology laboratory would struggle to complete the remaining uranium mining ecotoxicology research identified earlier in this review, let alone establish a commercial capability and secure a future based largely on externally-funded projects. While it is theoretically possible that the uranium mining research could be completed, with the required research simply equating to a check-list of experiments for completion, the

sub-optimal working environment that would result (due to slow progress, realities and/or perceptions of poor senior management support and direction, insecure futures, etc) would almost certainly result in staff losses and further delays and setbacks to the program.

Requirements

From this review, it is clear that there is a need for ***eriss***/SSD to maintain a strong commitment to the ecotoxicology laboratory, due to (i) the amount of uranium mining ecotoxicology research remaining, and (ii) the significant role that the ecotoxicology resource can play in the existing and future environmental protection of aquatic environments in northern Australia. In this context, this review regularly identified the need for a senior ecotoxicologist to undertake various roles and duties within the ***eriss*** Ecotoxicology program. These are summarised again below.

- To seek and gain external research funding for the ecotoxicology program;
- To provide the necessary ecotoxicology expertise and track record required on funding applications for ***eriss*** to have a realistic chance of securing funds;
- To act as a supervisor for Honours, Masters, PhD and other (eg summer scholarship) students that are vitally important in maintaining research progress;
- To coordinate, undertake and maintain efforts at developing collaborative linkages and proposals, and targeted capacity building;
- To develop commercial opportunities, design, oversee and manage commercial projects, and be the contact point for clients.

Most of these roles are associated with high level management responsibilities, higher than those normally expected of an EA6 level scientist, for which there was a vacant position. Although a lower level of research appointment (eg EA6 level) could assist with current research activities, it would be unlikely to come with the experience, network and publication record required to secure research funding as well as develop the commercial aspects. Given this, prior to the completion of this review, a formal recommendation was made to the Director, ***eriss***, and the Supervising Scientist for the appointment of a senior ecotoxicologist at the Senior Research Scientist (SRS) level. This recommendation, which is provided at Appendix J, was accepted and a position was created, advertised and filled for a 3 year term in late 2003 (also see Appendix J for final Position Profile). In line with SSD's need to identify and implement a strategic direction for its continued operation post-uranium mining, the position was given a broader role than just the management of the ecotoxicology program, also having responsibility of facilitating and coordinating the scientific aspects of the Division's external research and commercial assessment program.

In addition to the above and at the same time, it was recommended that the Laboratory Technician position (PN352; Suthidha Nou) be extended for a 12 month period from its expiry date of 6 January 2004. The need for such a commitment was also accepted, although given Departmental restrictions on the maximum length of contract not requiring press advertising, the position was extended for a further 6 months, to the end of June 2004. Whilst this was a positive move, in no way would it enable the completion of the uranium mining ecotoxicology research. Reflecting an acknowledgment by senior management of this requirement, a proposal to advertise position PN352 as an ongoing appointment was approved in May 2004, just prior to the publication of this review (Appendix K).

In summary, in order for the ecotoxicology laboratory to operate with sufficient efficiency and productivity, it is considered essential that the staffing allocation be maintained at a minimum of the two laboratory-based personnel and senior ecotoxicologist. The ecotoxicology research program should also look to utilise students to undertake specific projects of interest and sufficient relevance to the uranium mining research, but that are considered lower priority (eg the proposed postgraduate project assesseing the influence of DOM on the toxicity of uranium and other relevant metals – see Recommendation 4). If this level of commitment is maintained, it is envisaged that the uranium mining ecotoxicology research would be completed by mid 2005.

Beyond the uranium mining ecotoxicology research, it is expected that similar minimum staffing resources (ie one senior ecotoxicologist and two laboratory-based personnel) would be required to maintain a laboratory focusing on externally-funded research, for which students would play an even greater role than present, and commercial toxicity testing, for which fluctuating demand would necessitate added flexibility in staffing levels.

Recommendation

42. That the Ecotoxicology research program staffing allocation be maintained, at a minimum, at two laboratory-based personnel and a senior research ecotoxicologist.

Training and development

Training needs are always present and usually dictated by the requirements of specific projects and/or broader program themes. Training in the standard suite of software applications (eg Microsoft Office suite of applications) is available for all SSD staff at any time of the year. Other training requirements that are relevant to the ecotoxicology laboratory staff are briefly discussed below.

Toxicity testing protocols

As mentioned above, one of the existing laboratory staff, Suthida Nou, is still undergoing training in a number of the toxicity testing protocols. As of January 2004, she had been fully trained in the *M. macleayi* (acute and chronic) and *H. viridissima* tests, partially trained in the *L. aequinoctialis* and *M. mogurnda* tests and yet to be trained in the *Chlorella* sp. and *A. cumingi* tests. The other staff member, Alicia Hogan, is fully competent in all protocols including the partially developed *A. cumingi* reproduction test.

Experimental design and statistics

Both laboratory staff have expressed a desire to undertake additional training in experimental design and statistics, with one of these, Alicia Hogan, having taken the initiative and enrolled in one such course through CDU for the first semester of 2004. In addition, it is expected that a statistics course will be held at SSD in 2004, possibly by CSIRO/Melbourne University statistician, Professor David Fox, or Griffith University Ecology lecturer, Dr Jean-Marc Hero. All ecotoxicology laboratory staff should attend such a course if it proceeds.

Ecological risk assessment

Formal and in-house (ie on-the-job) training in ecological risk assessment techniques would be extremely useful for the professional level staff of the ecotoxicology laboratory. In-house training can be provided by the Ecological Risk Assessment Program Leader, Dr Peter

Bayliss. Other relevant training and development could be achieved by interacting and collaborating with other risk assessment groups in Australia (eg Monash University Water Studies Centre, CSIRO Land and Water Environmental Contaminants Directorate).

Commercial toxicity testing

With regards to commercial toxicity testing, the laboratory staff may require training in additional QC procedures, such as those required for a reference toxicity testing program. Project management skills of the professional staff are probably sufficient, providing that the discussion and relevant recommendations (eg Recommendation 27) provided in the Quality Assurance and Quality Control section of *Commercial Ecotoxicology*, above, are taken into consideration. In particular, there needs to be an ability to accurately cost commercial projects.

Recommendation

43. That further training of the Ecotoxicology research program staff be sought in several key areas, notably toxicity testing protocols, experimental design and statistics, ecological risk assessment and aspects of commercial toxicity testing, as relevant to their level and duties.

Concluding remarks

One of the key tasks of the review was to determine the extent of uranium mining ecotoxicology research (ie research relating to the aquatic toxicity of contaminants associated with uranium mining in the ARR) remaining to be completed by the ***eriss*** ecotoxicology laboratory. Although much research has been done over the past 15 years on the toxicity of uranium to local aquatic organisms, there are still some key unanswered questions and uncertainties that require investigation. While it is not possible, or for that matter even necessary, to answer all questions associated with this issue, this review has identified what it believes are the key remaining issues that must be addressed. Similarly, although a PhD project has recently been completed on the ecological effects of $MgSO_4$ to the aquatic biota of Magela Creek, there still remains substantial experimentation to be completed in order to satisfactorily address the key issues. More recently, other contaminants have emerged as being of potential concern, namely manganese and ammonia, and thus, also require some level of investigation.

Thus, the assumption, previously articulated by both SSD/***eriss*** and ARRTC, that the uranium mining ecotoxicology work was approaching completion (with the possible exception of unknown, emerging issues), appears to have been somewhat premature. This review has clearly identified that there is still substantial ecotoxicology research required to fill key information gaps, and that this research would probably take a minimum of 18 months to 2 years to complete. This timeline is based on an assumption of a minimum staffing level of one senior ecotoxicologist and two laboratory-based personnel, with any reduction to this jeopardising the group's ability to complete the necessary research. It is noted that during the course of this review, a long-term commitment was made by senior management to this level of staff resources.

Another of the review's key tasks was to provide advice on the future strategic direction of the ***eriss*** ecotoxicology laboratory in terms of both future research directions and commercial opportunities. It is of great significance and relevance to note that over the past 20 years, The

Supervising Scientist and various collaborators have developed the most comprehensive suite of ecotoxicological testing protocols in Australia and possibly the world, for tropical freshwater species. The body of knowledge and ecotoxicological resource that has developed over this time represents a key asset for The Supervising Scientist; one for which every effort should be made to retain and utilise well beyond the completion of the uranium mining ecotoxicology research.

Thus, this review supports, but also elaborates on the recommendation of Bayliss et al (2003; Appendix A of this report) for the Ecotoxicology program to *Maintain status quo and expand commercially*. In supporting this, it is strongly recommended that, to the extent possible, the primary function of the ***eriss*** ecotoxicology laboratory remain one of research; that is, the generation of information, knowledge and understanding for the broader community on the risks and impacts of contaminants in tropical aquatic ecosystems. For this to be sustainable, the Ecotoxicology research program must establish effective linkages and collaborations with key research institutions, governments and industry, and subsequently begin to secure substantially-sized, externally-funded research projects within the next two years.

The ecotoxicology laboratory appears geographically, competitively and logistically (ie infrastructure, equipment, procedures) well-placed to secure and successfully undertake more commercial work. However, as with the uranium mining ecotoxicology research, this ability depends on adequate minimum staffing resources, as previously outlined. Additionally, communication with key consulting firms, State and Territory government departments and industries needs to be initiated and maintained in order to maximise opportunities.

The remaining uranium mining ecotoxicology research timeframe of approximately 2 years provides some lead-in time to plan and implement a strategy for the future of the ecotoxicology laboratory, targeting externally-funded, non-uranium mining research and commercial activities as recommended in this review. It is hoped that the recommendations arising from this review can be used as the basis for determining this strategy.

Finally, within reason and using good judgement, externally-funded research and commercial opportunities that arise for the ecotoxicology laboratory should be taken whether or not they fall within the directions and timeframes recommended by this review.

References

ACMER 2003. *A practical guide to the application of the Australian and New Zealand guidelines for fresh and marine water quality (2000) for the Minerals Industry*. Australian Centre for Mining Environmental Research, Kenmore, Qld.

ANZECC & ARMCANZ 2000. *Australian and New Zealand guidelines for fresh and marine water quality*. National Water Quality Management Strategy Paper No. 4, Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra.

ARRRI 1988. *Alligator Rivers Region Research Institute: Annual research summary 1987–1988*. Supervising Scientist for the Alligator Rivers Region, Australian Government Publishing Service, Canberra.

ARRTC 2002. ARRTC Summary record of 10th Meeting. September 2002, Alligator Rivers Region Technical Committee Secretariat, Darwin.

ARRTC 2003a. ARRTC Summary record of 11th Meeting. February 2003, Alligator Rivers Region Technical Committee Secretariat, Darwin.

ARRTC 2003b. Key Knowledge Needs. Draft 4.6.03. Alligator Rivers Region Technical Committee, Darwin. Unpublished paper.

Australian Oceanographic Data Centre 2003. Sea surface temperatures. Australian Oceanographic Data Centre, December 2003, http://www.aodc.gov.au.

Baird D 1996. A consultancy on Wetland Protection and Management, as requested by the Environmental Research Institute of the Supervising Scientist (***eriss***). Internal report 215, Supervising Scientist, Canberra. Unpublished paper.

Baird D, Finlayson CM & Camilleri C 1995. Ecological impact of contaminants on wetlands: Towards a relevant method of risk assessment. In *Wetland research in the wet-dry tropics of Australia. Workshop, Jabiru NT 22–24 March 1995*, ed CM Finlayson. Supervising Scientist Report 101, Supervising Scientist, Canberra, 242–246.

Bayliss P, Hogan A, Camilleri C, Boyden J & Walden D 2003. Ecotoxicity testing. Discussion Paper for the 11th Meeting of the Alligator Rivers Region Technical Committee (ARRTC), 17–19 February 2003.

Brown VM 1994. Report on current procedures used in the Aquatic Toxicology Laboratory of the Alligator Rivers Region Research Institute. Internal report 140, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.

Camilleri C, Markich S, van Dam R & Pfeifle V 1998. *Toxicity of the herbicide Tebuthiuron to Australian tropical freshwater organisms: Towards an ecological risk assessment*. Supervising Scientist Report 131, Supervising Scientist, Canberra.

Camilleri C, Hogan A & Overall R 2002. Biological toxicity testing of water from the Ranger Uranium Mine process water treatment pilot plant. Commercial-in-Confidence Report to ERA Ranger Mine.

Charles AL, Markich SJ, Stauber JL & De Filippis LF 2002. The effect of water hardness on the toxicity of uranium to a tropical freshwater alga (*Chlorella* sp.). *Aquatic Toxicology* 60, 61–73.

Darwin Harbour Advisory Committee 2003. *Darwin Harbour Regional Plan of Management*. Northern Territory Department of Infrastructure, Planning and Environment, Darwin.

DBIRD 2003a. Major Northern Territory operational mines: Second quarter 2003 (April to June) activities update. Department of Business, Industry and Resource Development, Darwin, NT.

DBIRD 2003b. Major Northern Territory mining proposals: Second quarter 2003 (April to June) activities update. Department of Business, Industry and Resource Development, Darwin, NT.

DBIRD 2003c. Major Northern Territory closed mines: Second quarter 2003 (April to June) activities update. Department of Business, Industry and Resource Development, Darwin, NT.

Eriksen RS, Nowak B & van Dam RA 2001a. *Copper speciation and toxicity in a contaminated estuary*. Supervising Scientist Report 163, Supervising Scientist, Darwin.

Eriksen RS, Nowak B, Mackey DJ & van Dam RA 2001b. Relationship between copper speciation and toxicity in marine algae. *Marine Chemistry* 74, 99–113.

ERA 2002. ERA Ranger mine annual environmental management report, 1st September 2001 – 31st August 2002. Energy Resources of Australia Ltd.

Finlayson M, Klessa D & Rippon GD (eds) 1994. ANCA consultancy report: Project DN11 - Toxicology of the herbicide AF100. Internal report 159, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.

Franklin N, Stauber J, Markich S & Lim R 1998. *A new tropical algal test to assess the toxicity of metals in freshwaters*. Supervising Scientist Report 133, Supervising Scientist, Canberra.

Hogan A 2003. Toxicity of Djalkmara Billabong water to local aquatic organisms: Pre-release biological testing for the 2002–2003 Wet season. Internal Report 422, August, Supervising Scientist, Darwin. Unpublished paper.

Hogan AC, van Dam RA, Markich SJ, McCullough C & Camilleri C 2003. Chronic toxicity of uranium to the tropical green alga Chlorella sp. for the derivation of a site-specific trigger value for Magela Creek. Internal Report 412, Supervising Scientist, Darwin.

Hogan A, Peck M & Kennett R 2004. Screening for endocrine disrupting compounds in Kakadu plunge pools. Internal Report 486, June, Supervising Scientist, Darwin. Unpublished paper.

Holdway DA 1992. Uranium toxicity to two species of Australian tropical fish. *The Science of the Total Environment* 125, 137–158.

Humphrey CL, Lewis BF, Brown I & Suggit JL 1995. ERISS Protocol for the creekside monitoring of Magela Creek waters: I. Freshwater snail, *Amerianna cumingii*, reproduction and survival test. Internal report 180, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.

Humphrey C, Templeman S, Camilleri C & Klessa D 1997. *Evaluation of rehabilitation options for Mount Lyell using whole-effluent toxicological tests on freshwater organisms*. Mount Lyell Remediation Research and Demonstration Program. Supervising Scientist Report 122, Supervising Scientist, Canberra.

Hyne RV 1991. Evaluation of environmental factors affecting cladoceran (*Moinodaphnia macleayi*) survival and fecundity. Internal report 45, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.

Hyne RV, Rippon Gd & Ellender G 1992. pH-Dependent uranium toxicity to freshwater hydra. *The Science of the Total Environment* 125, 159–173.

Hyne RV, Rippon GD, Hunt SM & Brown GH 1996. *Procedures for the biological toxicity testing of mine waste waters using freshwater organisms*. Supervising Scientist Report 110, Supervising Scientist, Canberra.

Johnston A 1994. Submission by the Office of the Supervising Scientist to the *Review of current Commonwealth environmental protection research needs in the Alligator Rivers Region*. Internal report 138, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.

Klessa DA 2000. *The chemistry of Magela Creek: A baseline for assessing change downstream of Ranger*. Supervising Scientist Report 151, Supervising Scientist, Darwin.

Lasier PJ, Winger PV & Bogenrieder KJ 2000. Toxicity of manganese to *Ceriodaphnia dubia* and *Hyalella azteca*. *Arch. Environ. Contam. Toxicol*. 38(3), 298–304.

Markich SJ 1998. Effects of biological and physicochemical variables on the valve movement responses of freshwater bivalves to Mn, U, Cd or Cu. PhD Thesis, University of Technology, Sydney, Australia.

Markich SJ & Camilleri C 1997. *Investigation of metal toxicity to tropical biota: Recommendations for revision of the Australian water quality guidelines*. Supervising Scientist Report 127, Supervising Scientist, Canberra.

Markich SJ, Brown PL & Jeffree RA 1996. The use of geochemical speciation modelling to predict the impact of uranium to freshwater biota. *Radiochimica Acta* 74, 321–326.

Markich SJ, Brown PL, Jeffree RA & Lim RP 2000. Valve movement responses of *Velesunio angasi* (Bivalvia: Hyriidae) to manganese and uranium: An exception to the free ion activity model. *Aquatic Toxicology* 51, 155–175.

Mueller SO, Kling M, Firzani PA, Mecky A, Duranti E, Shields-Botella J, Delansorne R, Broschard T & Kramer PJ 2003. Activation of estrogen receptor alpha and ER beta by 4-methylbenzylidene-camphor in human and rat cells: comparison with phyto- and xenoestrogens. *Toxicology Letters* 142(1–2), 89–101.

Orchard S 2000. The development of a rapid response toxicity bioassay based on the feeding rate of the tropical cladoceran *Moinodaphnia macleayi*. Internal Report 335, Supervising Scientist, Darwin. Unpublished paper.

Orchard SJ, Holdway DA, Barata C & van Dam RA 2002. A rapid response toxicity test based on the feeding rate of the tropical cladoceran *Moinodaphnia macleayi*. *Ecotoxicology and Environmental Safety* 53(1), 12–19.

Peck MR 2000. A site-specific tropical sediment toxicity test using *Chironomus crassiforceps* to investigate metal bioavailability in acid-sulphate sediments. PhD thesis, University of Stirling, Scotland.

Peck, MR., Klessa DA & Baird DJ 2002. A tropical sediment toxicity test using the dipteran *Chironomus crassiforceps* to test metal bioavailability with sediment pH change in tropical acid-sulfate sediments. *Environmental Toxicology & Chemistry* 21(4), 720–728.

Riethmuller N, Markich S, Parry D & van Dam R 2000. *The effect of true water hardness and alkalinity on the toxicity of Cu and U to two tropical Australian freshwater organisms*. Supervising Scientist Report 155, Supervising Scientist, Canberra.

Riethmuller N, Camilleri C, Franklin N, Hogan AC, King A, Koch A, Markich SJ, Turley C & van Dam R 2003. *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT.

Rippon G & leGras C 1993. Proceedings of a workshop on water flea diet and its effect on toxicity testing and uranium toxicity. Internal report 103, Supervising Scientist for the Alligator Rivers Region, Canberra. Unpublished paper.

Rippon G, Hunt S, Camilleri C & Bailey M 1998. Project SM14 – Biological toxicity testing of waters from a plunge pool used for recreational purposes. In *Environmental Research Institute of the Supervising Scientist Annual Research Summary 1992–1994: Draft Report (incomplete), July 1998,* Internal Report 291, Supervising Scientist, Canberra.

Rouleau C, Tjälve H, Gottofrey J & Pelletier E 1994. Effects of some chelating agents on the uptake and distribution of ^{54}Mn(II) in the brown trout (*Salmo trutta*). *Pharmacology and Toxicology* 74, 271–279.

Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B & Lichtensteiger W 2001. *In Vitro* and *in Vivo* estrogenicity of UV screens. *Environmental Health Perspectives* 109 (3), 239–244.

Schreurs R, Lanser P, Seinen W & van der Burg B 2002. Estrogenic activity of UV filters determined by an *in Vitro* reporter gene assay and an *in Vivo* transgenic zebrafish assay. *Archives of Toxicology* 76 (5–6), 257–261.

Semaan M, Holdway DA & van Dam RA 2001. Comparative sensitivity of three populations of the cladoceran *Moinodaphnia macleayi* to acute and chronic uranium exposure. *Environmental Toxicology* 16, 365–376.

Smith A 2001. Development and application of the Moinodaphnia macleayi feeding rate bioassay for rapid toxicity assessment. Internal Report 369, Supervising Scientist, Darwin. Unpublished paper.

Sprague JB 1969. Measurement of pollutant toxicity in fish: bioassay methods for acute toxicity. *Water Research* 3, 793–821,

Stern H, de Hoedt G & Ernst J 2003. *Objective classification of Australian climates*. Commonwealth Bureau of Meteorology, Melbourne, Australia, November 2003, www.bom.gov.au/climate/environ/other/koppen_explain.shtml.

Stubblefield WA, Brinkman SE, Davies PH, Garrison TD, Hockett JR & McIntyre MW 1997. Effects of water hardness on the toxicity of manganese to developing brown trout (*Salmo trutta*). *Environmental Toxicology & Chemistry* 16, 2082–2089.

Supervising Scientist 2002. *Supervising Scientist Annual Report 2001–2002*. Supervising Scientist, Darwin NT.

van Dam RA 1998. Expanding the role of ecotoxicology in the Australian wet-dry tropics. October 1998, Internal Report 304, Supervising Scientist, Canberra. Unpublished paper.

van Dam RA 2002. Derivation of a site-specific water quality trigger value for uranium in Magela Creek. In *Environmental Research Institute of the Supervising Scientist research summary 1995–2000*, eds J Rovis-Hermann, KG Evans, AL Webb & RWJ Pidgeon, Supervising Scientist Report 166, Supervising Scientist, Darwin NT, 171–176.

van Dam RA, Camilleri C & Turley CJ 1999. Preliminary assessment of petroleum hydrocarbons in water and sediment at Yellow Water, Kakadu National Park. *Australasian J. Ecotox*. 4(1), 61–68.

van Dam RA, Camilleri C, Turley C & Markich SJ 2001. Ecological risk assessment of tebuthiuron following application on northern wetlands. Internal Report 362, Supervising Scientist, Darwin. Unpublished paper.

van Dam RA, Humphrey CL & Martin P 2002. Mining in the Alligator Rivers Region, northern Australia: Assessing potential and actual impacts on ecosystem and human health. *Toxicology* 181/182, 505–515.

Williams SJ, Camilleri C & van Dam RA 1998. Assessment of the suitability of the black-striped rainbowfish (*Melanotaenia nigrans*) as a laboratory toxicity testing organism. Internal Report 272, Supervising Scientist, Canberra. Unpublished paper.

Appendix A *eriss* Ecotoxicity Testing Discussion Paper (Bayliss et al 2003)

Discussion Paper prepared by Peter Bayliss, Alicia Hogan, Caroline Camilleri, James Boyden & Dave Walden for the 11th Meeting of the Alligator Rivers Region Technical Committee (ARRTC) 17–19 Feb 2003

Ecotoxicity testing

Paper prepared in response to a request from the 10th Meeting of ARRTC (9–10 Sept 2002)

ARRTC request: what is the future of this work? Work on U and Mg will soon be completed, so what next? Laboratory facilities will be maintained for pre-release testing and other non-mining toxicants (eg herbicides & possibly pesticides). If ecotox research is scaled down, what are the resource implications?

Ecotoxicology has historically been a fundamental component of the Ecological Risk Assessment program within ***eriss*** and underpins the Wetlands Risk Management approach for the Alligator Rivers Region (ARR). Hence, it needs to be discussed and reviewed within the context of the ecological risk assessment approach discussed separately.

Background – current status quo of ecotoxicology within *eriss*

1. Jabiluka is in care and maintenance mode whilst Ranger will wind down over several years. Research focus will therefore shift towards rehabilitation issues. Hence, the need for a fully operational ecotoxicology unit within ***eriss*** will reduce over time.
2. In addition to research projects on the toxicity of individual constituents of waters at Ranger, a major reason to maintain the ecotox facilities at eriss has been our commitment to the annual pre-release testing of Djalkmara Billabong waters. However, Djalkmara Billabong will soon be consumed by the development of Pit 3 and such testing will no longer be necessary.
3. Most major work on U and Mg will soon be completed (say by the end of 2003–04 FY). Apart from manganese, our current ecotox research projects aim at refinements to existing knowledge rather than necessary new core knowledge.
4. However, the concentrations of uranium, sulfate and magnesium in RP1 have been increasing in recent years. Also, effluent from the proposed new water treatment plant for process water will probably be directed to RP1 and/or Corridor Creek through wetland systems designed to remove ammonia. There is a need, therefore, to consider ongoing toxicological assessment of waters leaving the Ranger mine site and entering the Coonjimba and Georgetown Billabongs.
5. There has been a substantial investment over time in intellectual capacity (e.g. highly trained lab staff, development of international best practice testing protocols) and infrastructure in ecotoxicology within eriss. The new ecotox facilities at SSD's new building adjacent to Darwin International Airport are apparently the best in northern Australia and the Asia-Pacific. Careful consideration needs to be given, therefore, to the future of ecotoxicilogical work at ***eriss***.

What are our options?

6. There are three basic options, each with associated advantages, disadvantages and resource implications, which are discussed below. These are:
   - closure;
   - maintain status quo; and
   - maintain status quo and expand commercially.

Closure

7. Closure is not considered an appropriate option because even during the wind-down phase of Ranger the Supervising Scientist will still need the capacity to react quickly to unexpected or emerging toxicological issues. For example, trends in manganese levels on Ranger have increased, necessitating examination of its toxicity at least and, depending on preliminary results, development of site-specific trigger values (TVs) to manage water quality. Outsourcing to southern ecotox laboratories may not be the best solution because the current site-specific ecotox expertise and capacity remains within ***eriss***.

Maintain status quo

8. Maintaining the current status quo is a viable option but sub-optimal with respect to paragraph 4 above and 8 below. Staffing levels for ecotox work are adequately matched to the current (FY 2002–03) work program (two technical staff at 50% of time & one full-time staff – ie two full-time staff). Without the acquisition of externally funded work we predict a reduction in the ecotox workload in FY 2003–04, requiring only one full-time staff to maintain the status quo. The additional full-time equivalent staff position for ecotox work will, therefore, be shifted to ecological risk assessment at the landscape level.

Maintain status quo and expand commercially

9. Maintaining the current status quo and expanding commercially is a viable and attractive option. Whilst previous water quality assessments in Australia have been limited to provision of simple lists of chemicals in water and associated 'safe levels', the new National Water Quality Management Strategy (2000, NWQMS) explicitly identifies ecotoxicology as the required method to assess water quality. Additionally, Trigger Values for toxicants are defined on the basis of ecotoxicological results. Public comment on the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC & ARMCANZ 2000) identified also a need for more ecotoxicity tests on tropical species.

10. Hence, given the points outlined in paragraphs 4 and 8 above, ***eriss*** is strategically positioned to capture commercial ecotox opportunities in the region's tropics, particularly across northern Australian. Note also that there is no Environmental Protection Authority (EPA) in the NT and, in collaboration with the NT Government (DBIRD, DIPE & OTD (Office of Territory Development)) and the NT University (now Charles Darwin University); there is an opportunity to fill that vacuum.

11. Potential ecotoxicity issues that could be address by an expanded ecotox facility at ***eriss*** are listed below.
    - Salinity toxicity in tropical freshwater environments
    - Regional herbicide & pesticide pollution
    - Risk to health of humans and wildlife of endocrine disruptors

- Effects of urban by-products and waste (eg urban stormwater, sewage)
- Marine pollution (eg gas/oil & other industry developments in Darwin Harbour). The ANZECC Interim Ocean Disposal Guidelines (1998) specify the need for assessment of impacts of sediments on test organisms.
- Other mining pollutants from other regions in the tropics.
- New chemicals imported into Australia now require ecotoxicological testing (National Registration Authority for Hazardous Chemicals).

12. Another commercial and public good opportunity involves training students (undergraduate & postgraduate) in tropical ecotoxicology through participation in course development, teaching and supervision of postgraduate students (eg via collaborative ARC–Linkage grants). Most postgraduate training in Australian Higher Education for environmental assessments has adopted the physico-chemical approach. Training in tropical ecotoxicology could be facilitated via the NCTWR involving the three core university partners (UWA, JCU & CDU).
13. However, whilst we have excellent staff and infrastructure capability for commercial ecotox work we don't have the capacity. One option to overcome our critical mass problem is to develop a 'Northern Australia Ecotoxicology Centre' as part of a proposed "National Centre for Ecotoxicology". George Lukacs (James Cook University, Australian Centre for Tropical Freshwater Research) and Rick van Dam (now with Sinclair Knight Merz) proposed the former option via funding from AusIndustry Major National Research Facilities (& was to be facilitated through the NCTWR). Derek Eamus (UTS) and his southern collaborators proposed the latter option via a Systemic Infrastructure Initiative. Both these initiatives would need to be revived and pursued vigorously if this option is adopted.
14. Additionally, strong local partnerships with the NT Government and NT University need to be developed. In particular, with the NT Office of Territory Development (via its Tropical Knowledge Strategy), NT DBIRD (via its Strategic Mining Assessment Initiative), and the NTU Environmental Chemistry Laboratory (via Associate Professor David Parry). Similarly, we need to develop stronger links with local communities, particularly indigenous communities in remote areas with mining interests. Strong partnerships could be developed also with industry, government and local communities in WA and Qld. The Lake Argyle diamond mine in WA, for example, has an $MgSO_4$ problem which may require derivation of a TV to manage mine waste water.
15. If this option were pursued then we would need to increase our current staff capacity via one of the following.
    - Employ a full-time ecotoxicologist at an EA6 level (postdoc). This person could drive a revamped ecotoxicology unit within the Ecological Risk Assessment program with the twin goals of maintaining the capacity of the Supervising Scientist to respond to unexpected mine-related toxicity issues in the ARR and to seek new opportunities to grow. This position will: maximise the returns on our substantial infrastructure investment in a new ecotox laboratory; capitalise on current high staff capacity; develop critical partnerships (especially with universities & the potential to provide postgraduate training); and undertake commercial toxicity testing (including whole effluent toxicity testing & toxicity identification evaluation).
    - Outsource our opportunities to grow. For example, contract Rick van Dam from Sinclair Knight Merz (SKM) to help develop new commercial opportunities in ecotoxicity testing for ***eriss***.
    - Some combination of the above.

Recommendations

16. We recommend that the '***maintain status quo and expand commercially***' option be adopted as the optimal approach given our current capacity with respect to staffing and infrastructure, and assuming that there really are emerging commercial opportunities in tropical ecotoxicology to capture. To verify that the latter is in fact true and low risk, an assessment of market potential needs to be undertaken. This option also satisfies the need of the Supervising Scientist to provide assurance to the public, and in particular ARR stakeholders, that he has the capacity to react swiftly to unexpected pollution issues during the Ranger wind-down phase and, in the event that the JMA proceeds.

17. If ecotoxicology becomes part of core business again at ***eriss*** then there is a need to undertake a desktop review of the efficacy of traditional ecotoxicological models used in ecological risk assessment with a view to enhancing their application. In general, the ecological relevance of such models need to be increased (eg via supplementary mesocosm experiments &/or incorporation of life history models to fill critical gaps in ecological knowledge), and they need to be better underpinned by more robust statistical concepts of variability and uncertainty (Fox 1999). Additionally, there is scope to employ Bayesian statistics, which permits use of all available information in model selection (Hilborn & Mangel 1997). The general concerns summarised by Newman et al (2000) with respect to use of community ecotoxicity models, and the more specific concerns raised by Chapman et al (1996) with respect to use of NOECs, should be considered in any assessment.

References

ANZECC 1998. *Interim ocean disposal guidelines*. Australian and New Zealand Environment and Conservation Council, December 1998.

ANZECC & ARMCANZ 2000. *Australian and New Zealand guidelines for fresh and marine water quality*. National Water Quality Management Strategy Paper No 4, Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand, Canberra.

Chapman PM, Caldwell RS & Chapman PF 1996. A warning: NOECs are inappropriate for regulatory use. *Environ Toxicol Chem* 15 (2), 77–79.

Fox DR 1999. Setting water quality guidelines – A statistician's perspective. *SETAC News* 19 (3), 17–18.

Hilborn R & Mangel M 1997. *The ecological detective*. Princeton University Press, Princeton NJ.

Newman MC, Ownby DR, Mezin LCA, Powell DC, Christensen TRL, Lerberg SB & Anderson B-A 2000. Applying species sensitivity distributions in ecological risk assessment: assumptions of distribution type and sufficient number of species. *Environ Toxicol Chem* 19, 508–515.

Appendix B Summary of uranium toxicity to local aquatic species in Magela Creek water

Species	pH	Test Endpoint	Toxicity estimate (µg U L^{-1})	Reference
Chlorophyta				
Green alga (*Chlorella* sp.)				Hogan et al (2003)
Cnidaria				
Green hydra (*Hydra viridissima*)	6.5 ± 0.2	96 h population growth	160 (NOEC) [a] 190 (LOEC)[b]	ARRRI (1988)
	6.4 ± 0.3	120 h population growth	150 (NOEC) 200 (LOEC) (wet season)	Hyne et al (1992)
	6.5	120 h population growth	150 (LOEC) (dry season)	Hyne et al (1992)
Pink hydra (*Hydra vulgaris*)	6.4 ± 0.3	120 h population growth	550 (LOEC) (wet season)	Hyne et al (1992)
	6.5	120 h population growth	400 (LOEC) (dry season)	Hyne et al (1992)
Mollusca				
Mussel (*Velesunio angasi*)	5.5	48 h Duration of valve opening	367 ± 24 (LC_{50})	Markich et al (2000)
Crustacea				
Water flea (*Dadaya marcops*)	6.6 ± 0.1	24 h survival	1254 (LC_{50})[c] (923-1660)	Bywater et al (1991)
Water flea (*Diaphanosoma excisum*)	6.6 ± 0.1	24 h survival	1140 (LC_{50}) (787-1570)	Bywater et al (1991)
Water flea (*Latonopsis fasciculata*)	6.6 ± 0.1	24 h survival	467 (LC_{50}) (365-593)	Bywater et al (1991)
Water flea (*Moinodaphnia macleayi*)	6.6 ± 0.1	24 h survival	1470 (LC_{50}) (1210-1700)	Bywater et al (1991)
	6.5 ± 0.1	120 h reproduction	14-22 (NOEC) 19-30 (LOEC)	eriss (unpublished toxicity data; Tests 185, 199, 203, 207, 209)
	6.7 ± 0.1	96 h survival	10 (NOEC) 25 (LOEC)	Hyne et al (1993)
	6.7 ± 0.2	120 h reproduction	8-22 (NOEC) 20-42 (LOEC)	Semaan (1999)
	6.6 ± 0.1	20 h feeding rate	>290 (NOEC) nd (LOEC)	Orchard et al (2002)
M. macleayi (Bowerbird Billabong population)	6.9 ± 0.1	120 h reproduction	25-29 (NOEC) 36-49 (LOEC)	Semaan et al (2001)
Prawn *(Macrobrachium* sp.)	7.0 ± 0.1	96 h survival	> 5700 (LC_{50})	Giles (1974)

Appendix B cont.

Chordata – Osteichthyes				
Reticulated perchlet (*Ambassis macleayi)*	6.6 ± 0.1	96 h survival	910 (LC_{50}) (627-1230)	Bywater et al (1991)
Striped grunter (*Amniataba percoides*)	7.0 ± 0.1	96h survival	2850 (LC_{50})	Giles (1974)
Mariana's hardyhead (*Craterocephalus marianae*)	6.6 ± 0.1	96 h survival	1390 (LC_{50}) (935-1840)	Bywater et al (1991)
Marjorie's hardyhead * (*Craterocephalus marjoriae*)	7.0 ± 0.1	96 h survival	4850 (LC_{50})	Giles (1974)
Carp gudgeon (*Hypseleotris compressus*)	6.0 ± 0.4	96 h survival	7520 (LC_{50})	Cited in Markich & Camilleri (1997)
Spangled grunter (*Leiopotherapon unicolor*)	7.0 ± 0.1	96 h survival	4670 (LC_{50})	Giles (1974)
Black-striped rainbowfish (*Melanotaenia nigrans*)	6.6 ± 0.1	96 h survival	1940 (LC_{50}) (1410-1590)	Bywater et al (1991)
	6.6 ± 0.1	96 h survival	2160 (LC_{50}) (1740-2600)	Bywater et al (1991)
Chequered rainbowfish (*Melanotaenia splendida inomata*)	6.6 ± 0.1	96 h survival	3030 (LC_{50}) (2470-3740)	Bywater et al (1991)
	6.6 ± 0.1	96 h survival	3944 (LC_{50}) (2680-7490)	Bywater et al (1991)
	6.6 ± 0.2	96 h survival	1585 (LC_{50}) (1250-2000)	Holdway (1992)
	6.3 ± 0.2	168 h (7 d) survival	1790 (LC_{50}) 810 (NOEC) 1560 (LOEC)	Holdway (1992)
Purple-spotted gudgeon (*Mogurnda mogurnda*)	6.6 ± 0.1	96 h survival	1265 (LC_{50}) (950-1650)	Bywater et al (1991)
	6.6 ± 0.1	96 h survival	1665 (LC_{50}) (1280-2170)	Bywater et al (1991)
	6.4 ± 0.1	336 h (14 d) survival	1000 (NOEC) 2040 (LOEC)	Holdway (1992)
		336 h (+360 h post exposure) survival	502 (NOEC) 1000 (LOEC)	
	6.3 ± 0.2	168 h (7 d) growth	920 (NOEC) 1780 (LOEC)	Holdway (1992)
		168 h (+168 h post exposure) growth	<455 (NOEC) 455 (LOEC)	
	6.6 ± 0.2	96 h survival	1790 (LC50) (1385-2100)	Holdway (1992)
		96 h growth	640 (NOEC) 1240 (LOEC)	
	6.3 ± 0.2	168 (+168 h post exposure) survival	1640 (LC_{50}) (1120-2565)	Holdway (1992)
		168 h (+168 h post exposure) growth	1240 (NOEC) 2580 (LOEC)	

* This species is actually Mariana's hardyhead (Ivantsoff, Crowley & Allen) but was misidentified as Marjorie's hardyhead

Appendix B cont.

	6.6 ± 0.2	96 h survival	3750 (LC_{50}) (2580-4925)	Holdway (1992)
		168 (+168 h post exposure)	3078 (LC_{50}) (2580-3590)	
Blue eye (*Pseudomugil tenellus*)	6.6 ± 0.1	96 h survival	830 (LC_{50}) (570-1070)	Bywater et al (1991)

[a] NOEC, no-observed effect concentration.

[b] LOEC, lowest-observed effect concentration.

[c] LC_{50}, concentration at which there is 50% survival.

References

ARRRI 1988. *Alligator Rivers Region Research Institute: Annual research summary 1987–1988*. Supervising Scientist for the Alligator Rivers Region, Australian Government Publishing Service, Canberra.

Bywater JF, Banaczkowski R & Bailey M 1991. Sensitivity to uranium of six species of tropical freshwater fishes and four species of cladoceran from northern Australia. *Environmental Toxicology and Chemistry* 10, 1449–1458.

Giles MS 1974. Toxicity studies on aquatic organisms and grass-sedge communities in the Magela Creek area. In *The Alligator Rivers area fact finding study.* Four AAED Reports, eds PJF Newton, AAEC/E305, Australian Atomic Energy Commission, Sydney.

Hogan AC, van Dam RA, Markich SJ, McCullough C & Camilleri C 2003. Chronic toxicity of uranium to the tropical green alga Chlorella sp. for the derivation of a site-specific trigger value for Magela Creek. Internal Report 412, Supervising Scientist, Darwin.

Holdway DA 1992. Uranium toxicity to two species of Australian tropical fish. *The Science of the Total Environment* 125, 137–158.

Hyne RV, Rippon GD & Ellender G 1992. pH dependent uranium toxicity to freshwater hydra. *The Science of the Total Environment* 125, 159–173.

Hyne RV, Padovan A, Parry DL & Renaud SM 1993. Increased fecundity of the cladoceran *Moinodaphnia macleayi* on a diet supplemented with a green alga, and its use in uranium toxicity tests. *Australian Journal of Marine and Freshwater Research* 44, 389–99.

Markich SJ & Camilleri C 1997. *Investigation of metal toxicity to tropical biota: Recommendations for revision of the Australian water quality guidelines*. Supervising Scientist Report 127, Supervising Scientist, Canberra.

Markich SJ, Brown PL, Jeffree RA & Lim RP 2000. Valve movement responses of *Velesunio angasi* (Bivalvia: Hyriidae) to manganese and uranium: An exception to the free ion activity model. *Aquatic Toxicology* 51, 155–175.

Orchard SJ, Holdway DA, Barata C & van Dam RA 2002. A rapid response toxicity test based on the feeding rate of the tropical cladoceran *Moinodaphnia macleayi*. *Ecotoxicology and Environmental Safety* 53 (1), 12–19.

Semaan M, Holdway DA & van Dam RA 2001. Comparative sensitivity of three populations of the cladoceran *Moinodaphnia macleayi* to acute and chronic uranium exposure. *Environmental Toxicology* 16, 365–376.

Appendix C Uranium toxicity PhD proposal – 2000

Project Title

Toxicity of uranium to freshwater species of Magela Creek, Northern Territory: Derivation of a site-specific Trigger Value.

Supervisors

Associate Prof David Parry (NTU), Dr Rick van Dam (***eriss***)

Background

Uranium mining in the Magela Creek catchment, Northern Territory, has occurred for over 20 years. During this period, a comprehensive biological toxicity testing program has been developed, aimed primarily at assessing the toxicity of pre-release waste waters from the ERA Ranger Mine, with a specific emphasis on uranium toxicity (Humphrey et al 1999). The chronic toxicity of uranium has already been investigated for five local species, being a green alga (*Chlorella* sp.), cladoceran (*Moinodaphnia macleayi*), cnidarian (*Hydra viridissima*), and two fish (*Melanotaenia splendida inornata* and *Mogurnda mogurnda*), with no-observed-effect concentrations (NOECs) ranging from 18 to 810 μg L^{-1} (Camilleri et al in prep, Holdway 1992, Hyne et al 1993, Semaan 1999).

The revised NWQMS Water Quality Guidelines recommend a Trigger Value (TV) for uranium of 0.5 μg L^{-1} (ANZECC & ARMCANZ 2000). The TV is considered to be of *low reliability*, due to an inadequate toxicity database and the subsequent derivation of the value using the less-preferred safety factor approach. Given that the Magela Creek catchment is considered of high conservation/ ecological value, a *low reliability* TV is inadequate, and site-specific assessment is considered essential. In order to derive a *high reliability*, site-specific TV for uranium, chronic toxicity data for at least five local species from at least four taxonomic groups is required (ANZECC & ARMCANZ 2000). Using these data, the actual trigger value derivation process calculates a concentration that will protect x% of the species, which in the case of Magela Creek, would be 99% of species (ie it is classified as an area of high conservation/ecological value).

Although data currently exist for five local species, two of the species are fish, which are considerably less sensitive to uranium than the other species tested. In addition, the TV derived from the dataset of only 5 values carries much uncertainty. Thus, toxicity data from more species of invertebrates, and also aquatic plants, would help to improve the site-specific trigger value of uranium for Magela Creek.

Proposed methodology

Due to the necessary initial development of new, and modification of existing toxicity testing protocols, it is proposed that the project be carried out in several stages:

1. Assessment of the potential of new organisms as toxicity testing species;
2. Development of toxicity tests using local species considered appropriate and not previously assessed for uranium toxicity;
3. Assessment of the toxicity of uranium to local species and derivation of a revised site-specific Trigger Value for Magela Creek.

New organisms to be assessed for toxicity testing purposes would include species of isopods and mayflies (Ephemeroptera). Species currently used for toxicity testing purposes for which relevant procedures would need to be developed include duckweed (*Lemna aequinoctialis*) and the freshwater snail (*Amerianna cumingi*). It is anticipated that further toxicity assessment could be undertaken using four to five additional species.

In conjunction with the toxicity assessments, full chemical analyses will be undertaken in order to be able to predict the speciation of uranium using speciation models.

Project timeline

The project is expected to commence in March 2001. A full project proposal and literature review will be submitted by August 2001. The project will take approximately three years to complete (ie completion by March 2004).

Project outputs

The major outputs of the project include:

- the acquisition of several new toxicity testing protocols and an increased adaptability of several existing protocols;
- a scientifically sound site-specific trigger value for uranium in Magela Creek.

In addition, further understanding of uranium speciation and mechanisms of toxicity will be gained, while the whole process will serve as a model for similar processes elsewhere.

References

ANZECC & ARMCANZ 2000. Draft Australian and New Zealand Guidelines for Fresh and Marine Water Quality. National Water Quality Management Strategy.

Holdway DA 1992. Uranium toxicity to two species of Australian tropical fish. *The Science of the Total Environment* 125, 137–158.

Humphrey C, Thurtell L, Pidgeon R, van Dam R & Finlayson M 1999. A model for assessing the health of Kakadu's streams. *Australian Biologist* 12 (1), 33–42.

Hyne RV, Padovan A, Parry DL & Renaud SM 1993. Increased fecundity of the cladoceran *Moinodaphnia macleayi* on a diet supplemented with a green alga, and its use in uranium toxicity tests. *Australian Journal of Marine and Freshwater Research* 44, 389–99.

Semaan M 1999. Population variability in the response of *Moinodaphnia macleayi* to uranium and cadmium. Honours Thesis, Royal Melbourne Institute of Technology, Victoria.

Appendix D Summary of 1992–93 magnesium toxicity studies (compiled by Rick van Dam 1999)

Summary of magnesium (Mg) toxicity studies in the ARR

The only study undertaken on the toxicity of Mg to local aquatic species was an incomplete piece of research by Dr Greg Rippon in 1992–93 (Rippon et al 1998), summarised in the 1992–94 Annual Research Summary (Supervising Scientist 1998). Mg toxicity to the green hydra, *Hydra viridissima*, and the cladoceran, *Moinodaphnia macleayi*, was assessed. The study also examined the effect of the Mg:Ca ratio on Mg toxicity to *H. viridissima*. A summary of the results is presented below.[4]

Toxicity of Mg (added as $MgSO_4.6H_2O$)

A number of experiments were carried out assessing the toxicity of Mg to *H. viridissima* and *M. macleayi*. For *H. viridissima*, the NOEC for the tests ranged from 7.3 to 11 mg/L while the lowest-observed-effect concentration (LOEC) ranged from 12 to 17 mg/L. Fewer experiments were carried out for *M. macleayi*, with a NOEC and LOEC of 21 and 26 mg/L, respectively, being reported. All values represent chronic toxicity values, with the hydra test endpoint being 96-h population growth and the cladoceran test endpoint being 3 brood (~5 day) reproduction.

Thus, the lowest NOEC was 7.3 mg/L, at a Mg:Ca ratio was ~15, and the lowest LOEC was 12 mg/L, at a Mg:Ca ratio of 21.

Effect of the Mg:Ca ratio on Mg toxicity

In the above experiments (ie those strictly assessing the toxicity of Mg), no toxicity was observed below a Mg:Ca ratio of 21, over the range of Mg concentrations tested (ie ~3–30 mg/L).

Two experiments were carried out to better assess the effect of the Mg:Ca ratio on Mg toxicity to *H. viridissima*. To do this, the Mg concentration was kept relatively constant, while the Ca concentration was progressively decreased. The results are summarised in table D1.

For both tests, adverse effects of Mg (at 25–28 mg/L) on *H. viridissima* were only observed at the highest Mg:Ca ratios (ie 34 and 36).

Recommendation for Mg and Mg:Ca at 009

The research described above is insufficient to derive a high confidence trigger value for Mg or for a Mg:Ca ratio. Further research would need to be undertaken on more species and also assessing the toxicity of different Mg concentrations at different Mg:Ca ratios, or at least at Mg:Ca ratios characteristic of Magela Ck water at 009.

However, interim values can be derived, as described below.

Based on the lowest NOEC value for the most sensitive species (7.3 mg/L; *H. viridissima*), a trigger value of 0.73 mg/L can be derived by applying a safety factor of 0.1. Table D2 provides a summary of Mg, Mg:Ca and SO_4 upstream of Ranger and at 009 during the 1998–99 wet season. It is apparent that non-restricted release zone water releases do result in higher than background concentrations of Mg in Magela Ck at 009, with levels often exceeding the above trigger value. However, the Mg:Ca ratio downstream of Ranger (at 009), while

[4] Note that the summary of the results was taken from the project summary provided in IR291, as well as examining the original data sheets for each of the toxicity tests undertaken.

elevated from the upstream site is still much lower than the ratio used in the toxicity experiments by Rippon et al (1998). This further emphasises the need to know more about Mg toxicity at the Mg:Ca ratios characteristically found at 009.

Table D1 Summary of the effect of Mg:Ca ratio on Mg toxicity to *H. viridissima*

Experiment	Mg (mg/L)	Mg:Ca ratio	Response*
1	~25-28	1.7	~15% stimulation
		7.7	~15% stimulation
		10	No effect
		16	No effect
		36	~30% inhibition
2	~25-28	1.2	No effect
		5.8	No effect
		8	No effect
		13	No effect
		34	~40% inhibition

* Relative to the population growth of Control treatments (Control Mg:Ca ratio was 1.2; 0.69 ppm Mg : 0.59 ppm Ca)

Table D2 Summary data for Mg, Mg:Ca ratio and SO_4 upstream and downstream of Ranger during the 1998–99 wet season*

Parameter	Upstream Ranger			Downstream Ranger (009)		
	Mg (mg/L)	Mg:Ca	SO_4 (mg/L)	Mg (mg/L)	Mg:Ca	SO_4 (mg/L)
Mean	0.44	1.06	0.28	0.9	1.93	1.72
Std Dev.	0.17	0.39	0.15	0.6	0.86	1.64
Median	0.45	1	0.2	0.75	2	1.05
Range	0.1 – 0.8	0.3 – 1.7	0.1 – 0.7	0.1 – 2.4	0.3 – 3.2	0.2 – 5.8

* Data summarised from ERA (1999).

Given the above information, **a revised interim trigger value for Mg of 7.3 mg/L is recommended**, being the lowest NOEC recorded to date for local aquatic species (Rippon et al 1998). In addition, **an interim trigger value for the Mg:Ca ratio of 15 is recommended**. This corresponds to the Mg:Ca ratio at the lowest reported NOEC for Mg (Rippon et al 1998), and also to the approximate Mg:Ca ratio that would be recorded at 009 if the Mg concentration was to equal the trigger value (based on an historical approximate average Ca concentration at 009 of 0.4 – 0.5 mg/L).

Further research should be undertaken to assess the toxicity of Mg at a range of Mg:Ca ratios that are relevant to Magela Ck water at 009. In addition, worst case scenarios could also be assessed. Such assessments should be carried out for at least three aquatic species.

Toxicity of sulfate (SO_4^{2-})

In assessing the toxicity of Mg, Rippon et al (1998) also carried out an experiment assessing the effects of sulfate (SO_4^{2-}, added as Na_2SO_4). They reported no adverse effects to *H. viridissima* at SO_4^{2-} concentrations up to 100 mg/L. No higher concentrations were tested. In a study in South Africa, Goetsch and Palmer (1997) estimated the 96-h LC_{50} of Na_2SO_4 to the mayfly, *Tricorythus* sp. to be equivalent to 548 mg/L SO_4^{2-}. Thus, based on this limited

information for freshwater species, it appears that SO_4^{2-} will not be of direct toxicological concern at 009, particularly given the average SO_4^{2-} concentrations (for 1998–99) shown in table D2.

Insufficient data are available to derive a trigger value, and it is suggested that further ecotoxicological studies using local aquatic organisms be undertaken to better characterise the toxicity of SO_4^{2-}.

References

ERA 1999. Non-restricted Release Zone Water Release Report 1998/99. Energy Resources of Australia Ltd.

Goetsch PA & Palmer CG 1997. Salinity tolerances of selected macroinvertebrates of the Sabie River, Kruger National Park, South Africa. *Archives of Environmental Contamination and Toxicology* 32, 32–41.

Rippon GD, Hunt S, Camilleri C & Summerton J 1998. Toxicity of waters with artificially enhanced concentrations of constituents (seepage). In Environmental Research Institute of the Supervising Scientist Annual Research Summary 1992–1994: Draft Report (incomplete), July 1998, Internal Report 291, Supervising Scientist, Canberra.

Supervising Scientist 1998. Environmental Research Institute of the Supervising Scientist Annual Research Summary 1992–1994: Draft Report (incomplete), July 1998, Internal report 291, Supervising Scientist, Canberra. Unpublished paper.

Appendix E Manganese concentrations at selected sites around Ranger mine site

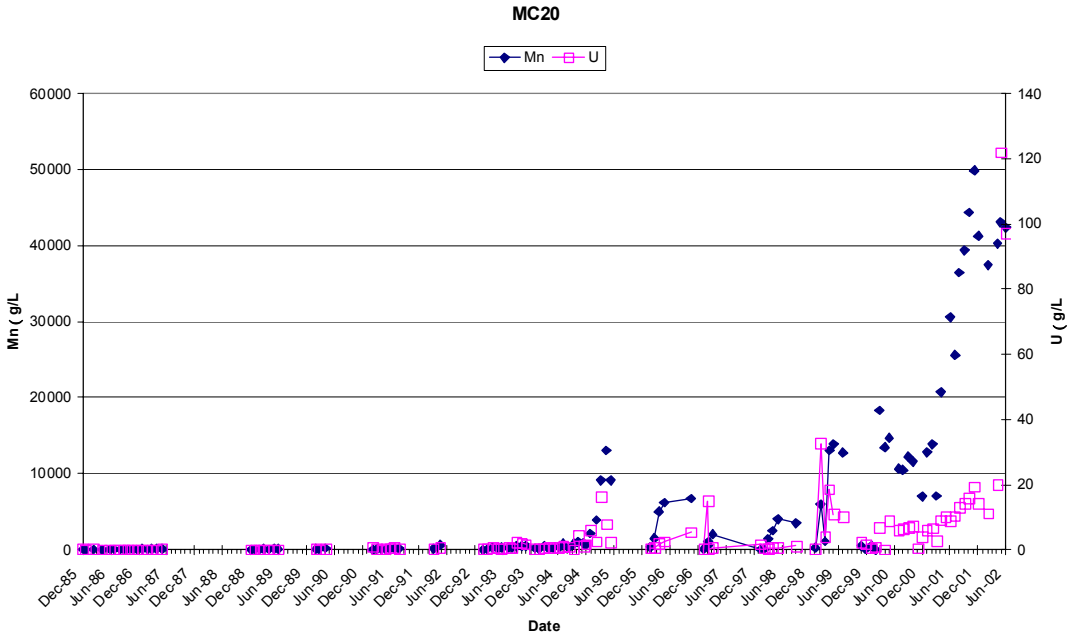

Figure E-1 Mn and U in groundwater from Bore MC20

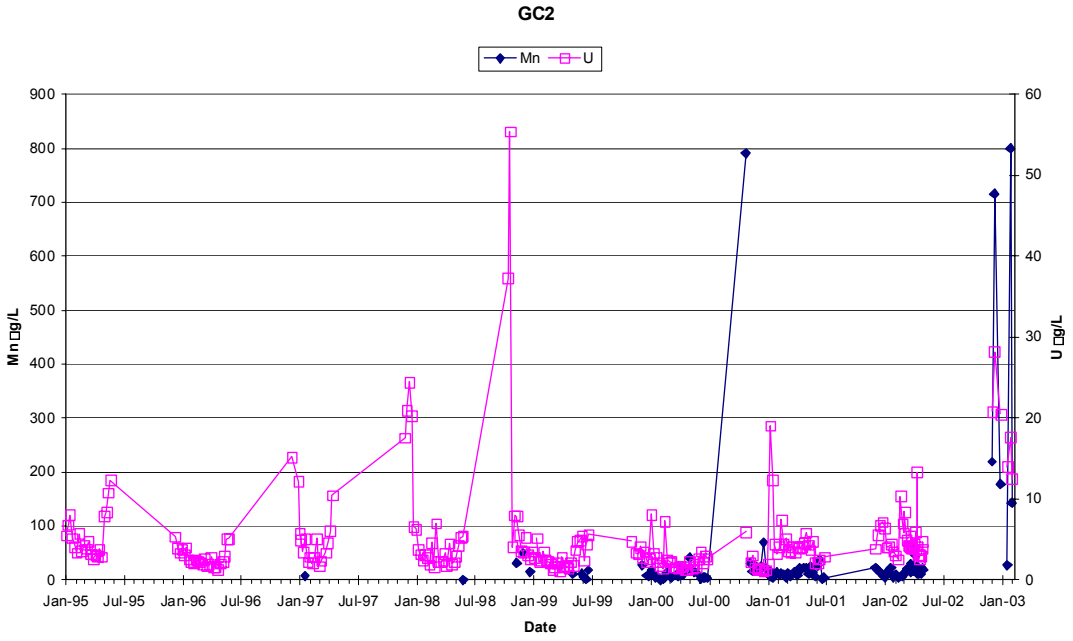

Figure E-2 Mn and U in Corridor Creek surface water

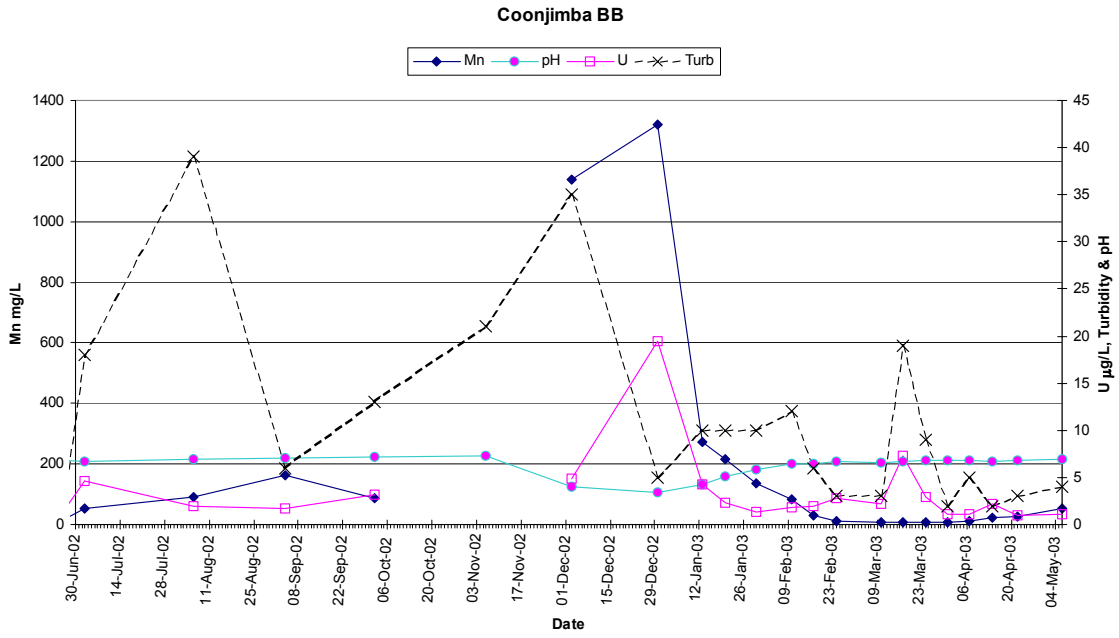

Figure E-3 Mn, U, pH and turbidity in Coonjimba Billabong surface water

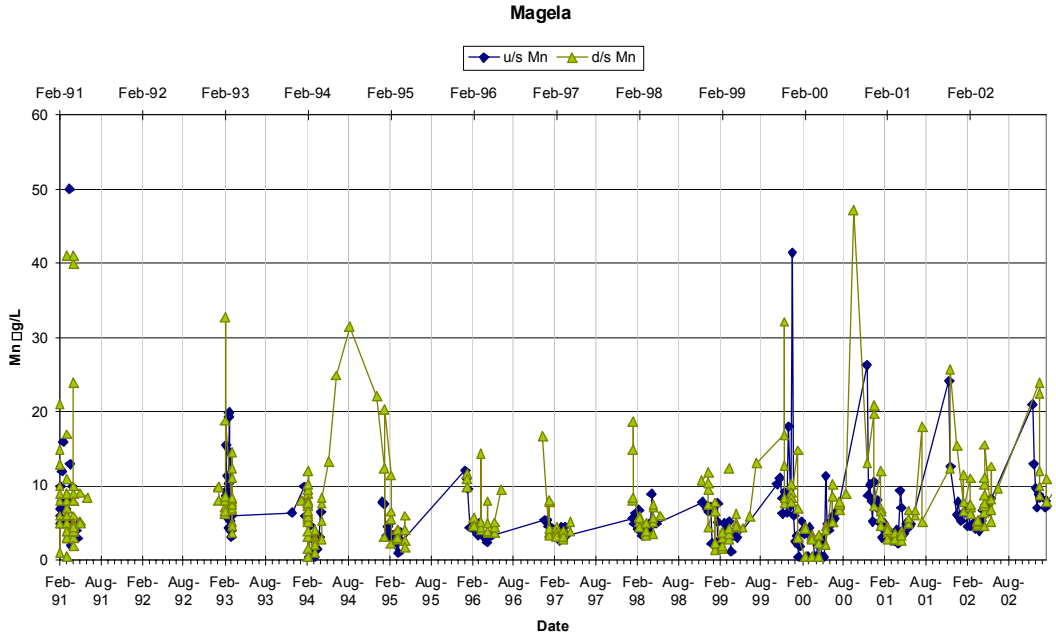

Figure E-4 Mn in Magela Creek surface water, upstream (u/s) and downstream (d/s; 009) of Ranger

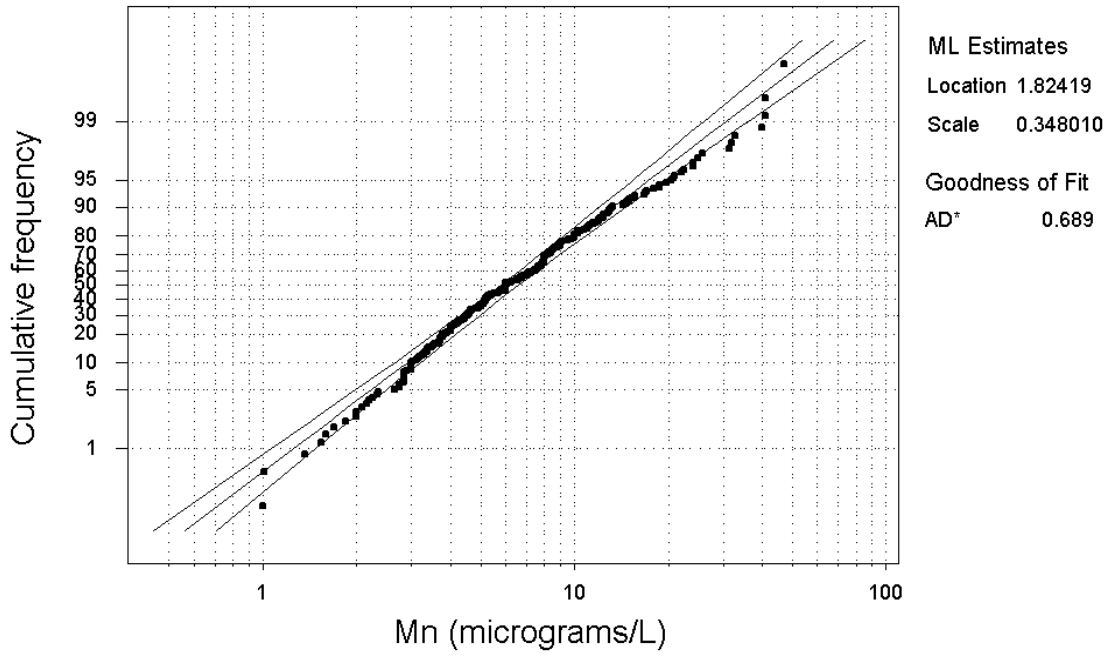

Figure E-5 Probability plot (loglogistic) for Mn downstream of Ranger (009; 1991–2003)

Appendix F Notes on a Northern Australian Ecotoxicology Centre (prepared by Rick van Dam, January 2002)

Background

One of the research needs to stem from public comment of the new Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC & ARMCANZ 2000) was the need for more ecotoxicity tests based on tropical species. Currently, the only comprehensive tropical ecotoxicology facility in Australia is located at the Environmental Research Institute of the Supervising Scientist (***eriss***) in Jabiru, NT. Even this facility has only a freshwater ecotoxicology capability, and given its core role of ensuring environmental protection from mining activities in the Alligator Rivers Region, is limited in the amount of additional ecotoxicology work it can undertake.

In early 2001, the idea of a Northern Australian Ecotoxicology Centre/Facility was raised by George Lukacs of the Australian Centre for Tropical Freshwater Research (ACTFR) at James Cook University (JCU), Townsville. Early discussions between George and myself revolved around the possibility of submitting an AusIndustry bid for major infrastructure funding. However, the timeline for submission of the bids, which needed to be extremely detailed and have written support of relevant industry sectors and government agencies, was too tight, and the opportunity passed.

Nevertheless, the concept of a Northern Australian Ecotoxicology Centre (referred to from herein as the Centre) still has great promise; it would be in line with the National Water Quality Management Strategy, and should attract both industry and government support. The following notes outline my thoughts on the scope, possible collaborators, and possible structure and capabilities of such a facility. They are intended merely to facilitate discussion in the hope that the concept can continue to progress towards some kind of reality.

Scope

The Centre would focus on the development and application of ecotoxicological and related techniques for tropical aquatic ecosystems. Perhaps the 'tropics' would incorporate the wet-dry tropics and the wet tropics, although I'm sure a more appropriate definition can be utilised. It would encompass freshwater, estuarine and marine ecosystems. Its primary focus would be research, but would include a commercial capability/arm.

The major driver of the ecotoxicological work undertaken by the Centre would be the ANZECC and ARMCANZ (2000) Water Quality Guidelines, in particular, the relevant research priorities identified by the Guidelines. This would include methodological development and focusing on data/research gaps. Attachment A lists some of the research priorities arising from the WQGs that I consider relevant to ecotoxicology in northern/tropical Australia.

A Strategic Plan should outline the major objectives of the Centre over the first few years, including its scope, developmental requirements (eg management, infrastructure, expertise), priority research themes, and expectations for commercialisation.

Centre Partners/Collaborators

The Centre would be a partnership between, at the very least, ***eriss***, and JCU's ACTFR. It is recommended that NTU's analytical/environmental chemistry group also be approached. This is Assoc Prof David Parry's group, which maintains one of the best reputations in Australia for ultra-trace level metal analyses, and is also involved in metal uptake and some ecotox-type research.

It might be considered beneficial and appropriate for other relevant research groups residing in the tropics/sub-tropics to also be a part of the Centre. This could include the National Research Centre for Environmental Toxicology (NRCET; Brisbane), where Barry Noller is Deputy Director, and Griffith University, where Heather Chapman is developing an ecotoxicology research base, currently with a major program on endocrine disruptors.

In addition, to assist with the commercial capability of the Centre, I would propose a formal link with the Sinclair Knight Merz ecotoxicology laboratory. The SKM ecotoxicology laboratory would be able to provide valuable assistance and support for establishing and maintaining a commercial testing facility under full QA/QC requirements.

Administration and Management

I have little idea of the legalities and requirements of a scheme such as this, but the following represents some initial thoughts. They should be treated in the context of my lack of understanding about these issues.

I would hope that the Centre could be administered and managed within the National Centre for Tropical Wetland Research (*nctwr*) management structure. If possible, the Centre would fall under the *nctwr* Board of Management and Advisory Committee. However, it might be appropriate for the Centre to have a separate Director to *nctwr*, and this person could be represented on the *nctwr* Board.

Structure and Facilities

As with the *nctwr*, staff would not 'belong' to the Centre, but would come under its banner for various ecotoxicological projects. This would include those staff of each partner organisation that are directly involved in ecotoxicological research. However, projects undertaken by the Centre could include other staff from the partner organisations, as well as scientists/specialists from other organisations, and postgraduate students.

The Centre could utilise existing ecotoxicology facilities of any of the partner organisations. There will probably be a need for new/additional facilities to be established at JCU, whilst the new ***eriss*** laboratories in Darwin could be extended to better accommodate a marine ecotoxicology capability. Related to this, marine ecotoxicology facilities could potentially be established at NTU, utilising or tapping into the salt water delivery systems already in place for the university's Aquaculture Centre.

Capabilities

The ecotoxicological capabilities of the partner organisations would need to be complementary to each other. For example, if the ***eriss*** ecotox capability centres around single-species freshwater and marine toxicity tests, another partner's capability could be focused towards multispecies (mesocosm) testing or sediment toxicity testing. Other important ecotox capabilities include *in situ* toxicity testing, ecological risk assessment, environmental chemistry (eg bioavailability and uptake studies), and biomarkers.

As stated earlier, the focus should be research and development, driven by priorities arising from the WQGs (see attachment A) and/or the relevant Advisory group(s). Where possible, the expertise and capabilities should be put to commercial use, in line with the WQGs and other relevant Guideline documents.

In addition, the Centre should look at ecotoxicology training opportunities, both within Australia and in tropical Asia.

In short, the Centre should have capability to:

- Develop single species and multispecies chronic toxicity tests for surface waters and sediments;
- Determine regional or site-specific guideline trigger values for toxicants, meeting the minimum requirements as detailed in the WQGs;
- Undertake research on the fate and effects of toxicants in tropical aquatic ecosystems;
- Provide relevant training in ecotoxicology, particularly for tropical ecosystems; and
- Undertake commercial toxicity testing, including whole effluent toxicity (WET) testing and toxicity identification evaluation (TIE).

A key aspect of the Centre is that it will be able to maximise its research capability by combining the complementary expertise of the various partners.

Funding

A key issue will be that of securing funding to expand existing and establish new infrastructure. George Lukacs is probably best positioned to comment on this issue. Discussions with George identified state/territory governments and relevant industry sectors as necessary supporters of the Centre, and therefore, potential funders.

As stated earlier, the initial thought was to apply for AusIndustry Major National Research Facilities funding (www.ausindustry.gov.au/), but there was insufficient time to prepare a proposal for this opportunity. There is no indication that a new funding round will be initiated.

Another opportunity, at least for JCU and NTU, could exist in the Systemic Infrastructure Initiative Scheme, a source of funding for universities that was initiated last year (I think), with further funding rounds to be run in 2002 and 2003. George Lukacs or David Parry might know more about this.

Attachment A – Relevant research priorities arising from the ANZECC & ARMCANZ (2000) Water Quality Guidelines

- Incorporating bioaccumulation into guidelines. There were few data available to use in the few available models, and the models themselves are deficient in several areas. It is not clearly known how and whether potentially bioaccumulating chemicals accumulate at the low ('no effect') guideline levels.
- Data gaps for water quality guidelines. There were many instances where the absence of data from just one taxonomic group forced the calculation of guidelines using less reliable and preferred methods or prevented calculation at all. The absence of algal and macrophyte data for many herbicides was of concern.

- Data are lacking on freshwater macroinvertebrates, a key component of New Zealand and Australian ecosystems.
- There needs to be considerably more information on the manner in which key chemicals interact with important water quality parameters such as temperature, pH, etc. The eventual aim should be to develop useable algorithms, similar to hardness algorithms for metals. There is little understanding on the issue of bioavailability of organic chemicals.
- Hardness algorithms need more data support using laboratory tests under controlled conditions. Algorithms are missing for vanadium, chromium (VI), aluminium and uranium.
- The interaction between organic chemicals and suspended matter is particularly poorly understood. Specifically designed experiments need to provide an understanding of both adsorption and desorption of key chemicals.
- Salinity effects require particular attention with the specific aim of using the decision scheme in estuarine environments.
- Sediment toxicity tests require further development (including chronic end-points), so as to provide the support, in a 'triad' context for chemical and field biological data.
- Further work is required to establish what is the minimum required dataset for use in the statistical distribution model (Campbell et al. 2000) to calculate trigger values.
- Field validation and assessment of guideline values for key chemicals is required in site specific situations.
- Future guidelines need to be able to assist in establishing if there is a relationship between the guideline figures for chronic exposure and short-term impacts from episodic exposure and, where appropriate, to provide short-term protection figures.
- There are too few recommended DTA protocols at present. More protocols need to be developed in order to sufficiently cover all geographical regions. Alternatively, protocols using more broadly applicable (ubiquitous) test species could be developed.
- Related to point no. 2, criteria for selection of DTA species (and end-points) need to be further developed.
- Sediment DTA methods need to be further developed (this is more related to SQGs).
- The next stage in sediment guideline development is the derivation of values based on Australian and New Zealand data. The cost and effort to undertake the necessary chemical analyses and toxicity testing on local sediments will be considerable. As a priority, a focussed program involving a number of key contaminants would be justifiable to see how locally-derived data compare with guidelines using overseas species.

References

Campbell E, Palmer MJ, Shao Q, Warne MStJ & Wilson D 2000. *BurrliOZ: A computer program for calculating toxicant trigger values for the ANZECC and ARMCANZ water quality guidelines*. Perth WA.

Appendix G Example Sample Tracking / Chain-of-Custody form (from EVS Environment Consultants)

CHAIN-OF-CUSTODY/TEST REQUEST FORM

EVS environment consultants
195 Pemberton Avenue
North Vancouver, BC
Canada V7P 2R4
Tel: (604) 986-4331
Fax: (604) 662-8548
www.evsenvironment.com

Please see instructions for completion on back. Shaded areas to be completed by EVS Laboratory upon sample receipt.

Client Name: _____ Client Contact Name: _____ Ship to: _____

Address: _____ Phone: _____

Fax: _____ Shipping Date: _____

Sampled By: _____ Attn: _____

Collection Date (dd/mmm/yy)	Time (24-h clock)	Sample Identification	1 Type of Each Sample	Material Safety Data Sheet Attached? (✓)	Sample Collection Method G = grab; C = composite	Number of Sample Containers x Volume of Sample Container (i.e., 1 x 20 L)	2 Sample Container Type by Code	Test(s) Requested						3 Notes? (e.g. preserved, saltwater, freshwater, may contain sewage)	Receipt Sample Temp. (°C)	4 Condition/Integrity	EVS Receipt Check List
For composite sample record date & time starting and ending																	EVS Project #:
																	EVS Workorder #:
																	Sample Storage Location: 4°C ❑ , Chemical ❑ , Other _____
																	Supporting documentation/other information attached if applicable.
																	1. Signatures & dates correct? Y N
																	2. Chain-of-custody fully completed? Y N
																	3. Containers arrived in good condition (unbroken)? Y N
																	4. Container labels completed (i.e., dates, ID's)? Y N
																	5. Container labels agreed with custody papers? Y N
																	6. Sample receipt temperature within acceptable range? Y N
																	7a. Sediment testing going to be initiated within 14 days? Y N
																	7b. If no, are samples under Nitrogen? If not, why? Y N
																	8. Chain-of-custody generated upon receipt ☐
																	9. Sample containers originate from EVS ☐

PO/Reference No.:	Comments/Instructions:
Project Title:	
Results Needed By:	

A) Released By:	Date:	B) Received by:	Date:	C) Released By:	Date:	D) Received by:	Date:
Company:	Time:	Company:	Time:	Company:	Time:	Company:	Time:
Courier name: _____		Shipping containers received secure? Yes No		Courier name: _____		Shipping containers received secure? Yes No	
Shipping containers secured by: Tape Straps Lock Other _____ (circle one)		Custody seals intact? Yes No N/A		Shipping containers secured by: Tape Straps Lock Other _____ (circle one)		Custody seals intact? Yes No N/A	
Custody seals used? Yes No				Custody seals used? Yes No			

1 Receiving Water (RW); Effluent (E); Elutriate (ELU); Sediment (SED); Chemical (CHEM); Stormwater (SW); Other (Please Specify)
2 Collapsible Carboy (CC); Glass Jar (GJ); Jerry Can (JC); Plastic HDPE (P); Plastic Bucket (PB); Other (Please Specify)
3 Please note any conditions the lab should be aware of for safety and storage concerns
4 Acceptable (A); Unacceptable (U). Please note specifics (e.g., broken, leaking, lid not on) under Comments/Instructions

Revision Date: Sept. 25, 2000

Distribution of copies: White, yellow, pink - accompany the shipment
Orange - retained by consignor (e.g., shipper)
Yellow - retained by consignee (e.g., receiver)
Pink - for use as needed
White - returned to consignor by consignee

Appendix G (cont.)

INSTRUCTIONS FOR COMPLETION OF CHAIN-OF-CUSTODY/TEST REQUEST FORM

1) Chain-of-Custody forms MUST accompany all samples shipped to and from EVS Consultants.
MUST be filled out with a black, ball point pen.
MUST be filled out completely, using clear language.
MUST exactly duplicate information on the sample labels.

2) If samples are packed in multiple shipping containers, use a separate form describing the contents of each shipping container.

3) If samples will be sent to multiple destinations, use a separate form for each set of samples. For example, if samples will be submitted to EVS for bioassay testing and to an analytical laboratory for chemical analyses, document each set of samples separately and include both forms in the shipment.

4) Distribution of copies: Retain orange copy for your records; white, yellow and pink copies accompany shipment.

CLIENT NAME:	The company/organization who will receive the final report.
ADDRESS:	Where the report will be sent (discharger).
CLIENT CONTACT NAME:	Person(s) to whom the report will be sent and all testing questions and comments should be directed.
PHONE AND FAX:	Numbers for client contact person.
SAMPLED BY:	Name or initials of the person(s) who actually collected the samples.
SHIP TO:	List the final destination of samples with full company name and address.
ATTN:	List a contact person at the final destination who is expecting the shipment. Please advise destination they will be receiving samples.
SHIPPING DATE:	Date samples began the shipping journey to final destination (the" released and received by" section will clarify any stop overs during transit).
COLLECTION DATE:	List the sample collection date using the format of "**01 Jan 99**" not "Jan 1/99" or "01/01/99".
TIME:	Use the 24-h clock (i.e., 0930, 1430) to record sample collection time.
SAMPLE IDENTIFICATION:	This must be identical to the information on the label. This is especially important since sample IDs are often alphanumeric combinations that are easy to confuse. Please note outfall numbers if applicable.
TYPE OF EACH SAMPLE:	Indicate appropriate sample type from list on bottom of front page (Note 1).
MATERIAL SAFETY DATA SHEET:	Mark a check here if a MSDS is supplied in the shipment. MSDS is required for all chemical samples before testing will be initiated.
SAMPLE COLLECTION METHOD:	The method used for sample collection (eg., grab, composite), not analytical methods or test requests.
NUMBER OF SAMPLE CONTAINERS x VOLUME:	1x20 L is one twenty-litre container; 20x1 L is twenty one-litre containers. Sample containers should always be filled completely, with no headspace. If a container is not completely filled, please add a comment in the Notes section (eg. jar only half full, some head space left in collapsible).
SAMPLE CONTAINER TYPE BY CODE:	Indicate appropriate container type from the list on the bottom of the front page (Note 2). Note that HDPE is High Density Polyethylene; if your container is HDPE, it will be marked as such below the recycling symbol on most plastic containers.
TEST(S) REQUESTED:	Clearly indicate the test(s) to be performed on each sample, including duration and species when appropriate (eg. 10-d *Ampelisca*; 96-h Rainbow trout LC50). Do not list more than one test in the same column. For questions, please contact the laboratory performing the analyses.
NOTES:	Provide detailed comments concerning the samples (eg., freshwater, saltwater, preserved, container only half full). It is essential that any potential health or safety hazards be clearly indicated (i.e., may contain sewage, may be high in certain contaminants, may contain chemical formulation [see MSDS]).
PO/REFERENCE NO.:	Provide purchase order number, contract number or other reference number that may be included in the report.
PROJECT TITLE:	Provide study title to be included in the report.
RESULTS NEEDED BY:	Provide date by which data is needed. Please note for bioassay results the routine turnaround is 2 weeks for aqueous and 4 weeks for sediment tests, from test termination.
COMMENTS/INSTRUCTIONS:	Provide any supplementary instructions regarding testing (eg., whether pH is to be adjusted, whether grab samples are to be composited, whether specific concentrations are to be tested) or reporting.
RELEASED BY:	Legible signature of the person releasing the sample(s) (the consignor). If signature is not legible, please print the name below the signature. Indicate the company name, date (dd/mmm/yy) and time (24-h clock) samples were released.
COURIER COMPANY:	If a courier company is used to ship the samples, provide the full name of the courier company.
METHOD OF LOCKING SHIPPING CONTAINER:	How was the shipping container sealed shut? Circle appropriate method; write "None" if no locking mechanisms were used (note that tape and straps are **not** locks).
CUSTODY SEALS:	Have custody seals been used in this shipment? Circle Yes, No, or N/A.
RECEIVED BY:	Legible signature of the person receiving the sample(s) (the consignee). If signature is not legible, please print the name below the signature. Indicate the company name, date (dd/mmm/yy) and time (24-h clock) samples were received. Circle appropriate answer to questions.
SHADED AREA:	To be completed by the EVS Laboratory upon sample receipt.

Appendix H Summary of investigations into sources of contamination and actions taken (compiled by Alicia Hogan, November 2003)

Analysis undertaken	Information gained	Action taken/recommended	SSD explorer pathway to data file
Regular toxicity test chemistry	Elevated Cu (>1μg/L) and Zn (>2 μg/L) occasionally recorded in test waters in Jabiru however, are far more frequently observed in Darwin. Pb concentrations have been consistently low at both locations. Al concentrations are highly variable, however, are generally within the range observed in Magela Creek. Autoclaved test waters for *Lemna* testing frequently had Cu concentrations between 3–15 μg/L.	Further investigation into the source of contamination was initiated. Procedural blanks were introduced for all toxicity tests in addition to the regular Milli-Q blanks. Investigation into the need for sterile *Lemna* test media was undertaken and autoclaving step was eliminated from testing.	\\Ecotoxicology of the Alligator Rivers Region\Laboratory Issues\Data\Contaminants in lab waters 2001-2003.xls
Cu concentrations in Milli-Q and Magela Creek water samples stored for 4 days in various lab vessels	Darwin tapwater was identified as a likely source of Cu (290 μg/L). Filtration through Whatman #42 filter papers contributed 6.3 μg/L Cu to Milli-Q water. A range of lab storage/testing vessels contributed 1–4 μg/L Cu (eg silanised and unsilaised conical flasks, 5L bottles, petri dishes). No pattern was apparent. 0.1% dishwashing powder (Gallay Clean A) did not contain Cu. Both the green and clear non-powdered vinyl gloves used in the ecotox lab did not release Cu when dipped in 10% HNO_3. Filtered aquaculture water contained 7 μg/L Cu.	Extreme care was taken to ensure that tapwater never comes in contact with creek water, test solutions or test organisms (eg, lab technicians only wash their hands and benchtops with Milli-Q, worms for fish feeding are now rinsed only with filtered aquaculture water etc). Dishwasher was re-plumbed so that it only uses Elix (demineralised) water for all phases. Further investigation into the use of Whatman # 42 papers for creek water filtration.	\\Ecotoxicology of the Alligator Rivers Region\Laboratory Issues\Data\Cu in lab test vessels 070403 EL02258.xls

Appendix H (cont.)

Analysis undertaken	Information gained	Action taken/recommended	SSD explorer pathway to data file
Full metal scans on Darwin laboratory waters	Confirmed that Darwin tapwater was a likely contamination source (1040 μg/L Cu and 738 μg/L Zn in one sample). Sample bottle preparation method (soaking in fresh 5% HNO_3 bath followed by 5 RO and 5 Milli-Q rinses) is sufficient as only a small number came back with Cu, Zn or Al contamination. That the activated carbon filters are not fully removing metals from the aquaculture water. Metals such as Cu and Zn may be of concern in terms of chronic exposure to the gudgeons. Unnecessary to analyse for all metals as many were not detected at all over a 13 month period. Elix water is very pure!	Arranged for filter media to be replaced with high quality hospital grade activated carbon. Initiated weekly Cl_2 checks of filtered aquaculture water to act as an indicator of carbon media exhaustion. Continue with monthly metal analysis of aquaculture water, however, reduce the suite of metals reported to those detected regularly and/or of toxicological significance.	\\Ecotoxicology of the Alligator Rivers Region\Laboratory Issues\Data\Aquaculture water chemistry\chem lab water ALL RESULTS.xls
Jabiru tapwater full metal scans	Cu and Zn were lower in these samples than have been observed in Darwin tapwater, supporting the theory that Darwin tap water via the dishwasher may be the source of contamination. Low levels of Cu and Zn in blank.	Reviewed dataset for other cases of blank contamination. As occurrence seems quite uncommon, the current sample bottle preparation technique (see above) will be continued.	\\Ecotoxicology of the Alligator Rivers Region\Laboratory Issues\Data\Jabiru ecotox lab waters Oct 03 EL02766.xls
Direct comparisons of contamination in filtered and unfiltered waters	#42 filters may be contributing to the contamination problem however more data is needed to confirm.	More data will need to be collected before a conclusion is made. Initial enquiries indicate that it may be difficult to find a cleaner filtering method for the substantial volumes that we handle.	\\Ecotoxicology of the Alligator Rivers Region\Laboratory Issues\Data\#42 filtration_metal contam 2003.xls

Appendix I Laboratory maintenance charts

WEEKLY LABORATORY MAINTENANCE TASKS

Task	Monday	Tuesday	Wednesday	Thursday	Friday	Sat/Sun
Incubator temp check						
Demin system check-cond of RO and Milli-Q						
DO calibration and moisten sponge						
Conductivity calibration						
pH calibration and change probe soln						
Incubator cladocera						
Bowl cladocera (B2 day)	Day will vary					
Prepare brine shrimp cone						
Feed and clean outside hydra stock						
Feed and clean inside hydra stock						
Feed gudgeons and archerfish						
Check temperature of fish tanks/Cl test						
Innoculation of Chlorella and Lemna media						
Run Coulter Counter						
Dishwashing	AS REQUIRED					
Worm farm feeding and maintainance						

ANNUAL LABORATORY MAINTENANCE PROGRAM

Mark dates when each task is undertaken

Task	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Clean fish tanks (fortnightly) - Gudgeons												
- Archerfish												
Algal harvest media (monthly)												
Culture media (monthly)												
Clean aquaculture area (monthly)												
Collect and filter creekwater (when required)												
Detergent bath (as needed)												
Acid baths (2–3 monthly)												
Pipette calibration (6 monthly)												
Thermometer check (6 monthly)												
Balance calibration - Single point (monthly)												
- Repeatability (6 monthly)												
Balance service (annually)												

Annual Laboratory Maintenance Program (cont.)

Cladoceran food preparation (3–4 monthly)												
Algal slopes (6 monthly)												
Microscope service (annually)												
Laminar flow service (annually)												
Coulter counter service (annually)												
DTW samples for metal and TOC analysis												

Appendix J Recommendations for appointment of an SRS – Ecotoxicologist and contract extension for EA3 Laboratory Technician position

To: Arthur Johnston 23 September, 2003

Through: Max Finlayson

From: Rick Van Dam

Subject: Proposal for Ecotoxicology Positions

Doc name: minute_senior ecotox proposal **File ref:**

Arthur,

As my review of the ***eriss*** Ecotoxicology Program continues, it is becoming increasingly clear that the various constraints on the current activities, output and future of the program are due almost exclusively to the lack of staffing within the group.

In particular, a senior ecotoxicologist is required to manage the program's current workload and future research directions.

In addition, there needs to be a longer-term commitment to technical level staffing in order for progress to be made and outputs realised for current ecotoxicological core research, which is estimated to require another 12–18 months to complete (see Attachment A for further details). We can discuss this further if required.

Below I have summarised the key consequences associated with the lack of a senior ecotoxicologist and non-continuation of the existing technical officer temporary contract (PN352; ie Tida's position) beyond January 2004.

Table J1 Summary of consequences of insufficient level of staffing in Ecotoxicology Program

Lack of Senior Ecotoxicologist	Non-continuation of Technical Officer contract (PN352) beyond January 2004
Core Research	
Slowed progress on core research due to EA-4 Officer having to manage administrative, liaison & report writing tasks	Little if any progress on core research beyond January 2004
Insufficient expertise for data interpretation and subsequent impact on work to come	Difficulty in maintaining laboratory cultures
Insufficient expertise/resources for reporting and publication	
Non-core Research	
Limited ability to set and implement strategic direction of ecotoxicology program	Limited ability to expand capabilities of the ecotoxicology program to accommodate future research directions (eg marine ecotoxicology)
Limited ability to undertake commercial work due to lack of senior contact/project manager	Limited ability to undertake commercial work due to shortage of technical staff
Limited ability to communicate/liaise with Government and industry on relevant issues	Limited ability to undertake externally funded research due to shortage of technical staff
Limited ability to collaborate with other institutions and to apply for external research funding	
Reduced chances of gaining external research funding	
Limited ability to host postgraduate students due to lack of on-site supervisor	

It is clear that in its current state, the ecotoxicology program will struggle to complete the remaining core research related to Ranger let alone develop and implement a strategic direction for its future activities, be they research or commercial in nature. In effect, the key recommendations of the review I am currently undertaking will not be achievable unless an investment is made in the staffing level of the ecotoxicology program. The Attachment below outlines the draft recommendations of my review to date and highlights the amount of ecotoxicology core research still required.

Below, I have outlined the approximate costs of a 3 year appointment of a senior ecotoxicologist at the SRS level (figures supplied by Karl Dyason). Although a lower level of research apppointment could assist with current research activities, it would be unlikely to come with the experience, network and publication record required to secure research funding as well as develop the commercial aspects. Without this ability, the ecotoxicology program is unlikely to be viable within 18 months.

Table J2 Estimated maximum costs of a 3 year appointment of a Senior Ecotoxicologist at the SRS level

Year	Salary	Perf Pay	RLA	Super	Prod Super	Airfares	Total Direct Cost
1 (SRS 1.3)	84,394	8,000	3,230	10,956	1,962	5,008	113,550
2 (SRS 1.4)	89,268	8,000	3,230	12,080	2,163	5,008	119,749
3 (SRS 1.4)	93,285	8,000	3,230	13,060	1,866	5,008	124,449
						3 Year Total	**$357,748**

The position would have a key duty of securing external funding for the ecotoxicology program, through both research grants and commercial contracts. It is envisaged that a realistic and successful program outcome at the end of the three year appointment would comprise:

- Completion and peer-reviewed publication of the ecotoxicology core research;
- 1 – 2 large external research grants for strategic, collaborative projects;
- 2 – 3 Honours or postgraduate student projects; and
- An established commercial component of 3–4 small to medium contracts annually.

Rather than providing a similar summary for the continuation of the existing technical officer temporary contract (PN352), I would simply recommend that this position be extended for a 12 month period from its current expiry date in order to ensure the completion of the remaining ecotoxicology core research.

Key Duties of Senior Ecotoxicologist

Below I have provided a first draft of Key Duties of a senior ecotoxicologist. This could be finalised following discussions and feedback from you, Max and Peter.

Within the Ecological Risk Assessment Program, manage the ecotoxicology research program, specifically:

1. Ensure the successful completion, reporting and publication of the remaining ecotoxicology core research;
2. Develop and implement a strategic plan for the future of the ecotoxicology program including the establishment of research directions and commercial capabilities;
3. Liaise/communicate with other research institutions, Government agencies and industry in order to identify and progress collaborations and/or issues for research and commercial project opportunities;
4. Seek and gain external research funding for the ecotoxicology research program and for ecological risk assessment projects in general;
5. Manage ecotoxicology research projects and commercial contracts;
6. Manage the staff and other resources of the ecotoxicology laboratory; and
7. Actively participate in ecological risk assessment projects across the Institute.

Recommendations

1. That a senior ecotoxicologist be appointed for a period of 3 years at the SRS level.
2. That the existing technical officer temporary contract for PN352 be extended for a 12 month period from its current expiry date (6 January 2004).

Your consideration of this would be much appreciated.

Rick

Appendix J (cont.)

Environment Australia

Department of the Environment and Heritage

Position Profile

Reference Number:	**Date Approved:**
Job Title:	Senior Research Scientist – Ecotoxicology
Designation:	EA Senior Research Scientist
Division:	Supervising Scientist Division
Branch:	Environmental Research Institute of the Supervising Scientist (***eriss***)
Section:	Ecological Risk Assessment
Subsection:	N/A
Location:	Darwin
State:	Northern Territory
Supervisor:	Dr Peter Bayliss

3.

Duty Statement

1. Develop and implement a strategic plan for the future of the ecotoxicology program including the establishment of future research directions and commercial capabilities.
2. Manage the projects, staff and resources of the ecotoxicology research program, and ensure the successful completion, reporting and publication of ecotoxicology core research.
3. Develop new research projects and commercial contracts in ecotoxicology and ecological risk assessment.
4. Seek and gain external research funding for new ecotoxicology and ecological risk assessment projects.
5. Initiate and contribute to broad ecological risk assessment projects across the Institute.
6. Facilitate the development of partnerships with research institutions, Government agencies, industry and other stakeholder groups in line with the Division's strategic research and commercial directions.
7. Work with the Division's senior staff to facilitate and coordinate the scientific aspects of the Division's research and commercial assessment program.

4.

Selection Criteria

1. A PhD or equivalent in Ecotoxicology.
2. Extensive experience in the research and commercial sectors, preferably in the fields of Ecotoxicology and Ecological Risk Assessment.
3. High level verbal and written communication skills including a demonstrated publication record.
4. High level ability to manage people, projects and programs to achieve high quality outcomes including the ability to ensure the principles of equity and diversity, workplace participation and occupational health and safety are preserved and practiced in the work area.
5. Demonstrated leadership abilities including coordination and leadership of multi-disciplinary teams.
6. Sound knowledge of and strong links with relevant Territory, State and Commonwealth Government agencies.
7. Extensive knowledge of current environmental issues in Australia including differences between northern and southern regions.

Note: Applications will not be acknowledged on receipt. Only shortlisted applicants will be contacted regarding the next phase in the selection process.

Appendix K Request for continuation of EA3 Laboratory Technician position (PN352)

To:	Arthur Johnston	5 May, 2004
Through:	Max Finlayson	
From:	Rick Van Dam	
Subject:	Extension of contract for PN352 (Suthidha Nou)	
Doc name:	minute_pn352 continuation	
cc:	Peter Bayliss	**File ref:**

Arthur,

Suthidha Nou's contract (PN352) expires at the end of June 2004. As I have indicated in my review of the ***eriss*** Ecotoxicology program (in final draft), it is imperative that at least 2 laboratory-based positions are maintained in the ecotoxicology laboratory (see extracts from ecotoxicology review at Attachment A). This requirement is based on both the need to complete the remaining core ecotoxicology research and to ensure the capacity to undertake effective commercial ecotoxicity testing, now and in the future. With a senior ecotoxicologist now appointed within the program, the two existing technical staff have been able to appropriately maintain the laboratory whilst also making steady progress on toxicity testing for core research projects. To illustrate, in the 7 months since late October 2003, when the laboratory began to again function fully with two dedicated technical staff and a senior ecotoxicologist, 21 toxicity tests have been completed. This compares to 6 completed toxicity tests in the prior 7 month period.

From an operational perspective, three options exist:

- renew the contract for PN352;
- do not renew the contract for PN352 and instead, re-assign an existing staff member to the ecotoxicology laboratory; or
- do not renew the contract for PN352 or re-assign an existing staff member.

Working back, option 3 is not realistic as it would leave no capacity to complete core research or undertake commercial activities. The second option, whilst probably preferred from a budgetary perspective, is not preferred from my perspective, primarily because I do not believe that the potential staffing options could result in the maintenance of the existing level of productivity. I am happy to elaborate upon this in follow-up discussions. From my perspective, the first option is preferred and the one I am requesting, as it provides continuity and enables the associated productivity to be maintained. Continuity and productivity are key requirements for being able to successfully complete the remaining core research in a timely manner and effectively develop a commercial ecotoxicity testing capability.

Given that by the end of June the contract position will have been occupied on an unadvertised basis for 12 months, I understand that a new contract/position would need to be appointed through the appropriate advertising process. In the event such

approval is granted, we will need to consider whether the contract be advertised on an ongoing or non-ongoing basis. Considering the preferred strategic direction of the ecotoxicology program (ie a freshwater and marine tropical ecotoxicology research and viable commercial ecotoxicity testing capability/component), an ongoing basis is preferred.

Recommendation

That the existing technical officer temporary contract for PN352, currently occupied by Suthidha Nou, and due to expire at the end of June 2004, be advertised for renewal for a substantial time period, preferably ongoing.

I would greatly appreciate it if you could consider this issue as a matter of priority, such that we can be in a position to make any necessary arrangements within an appropriate timeframe.

Rick