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Investigation of metal toxicity to tropical biota

Recommendations for revision of the Australian water quality guidelines

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Executive summary

The current Australian water quality guidelines for the protection of aquatic life are based predominantly on toxicological data derived from North American temperate studies. This is due to a lack of relevant toxicological data for Australian species. Thus, there is a clear requirement to assess the suitability of the current guidelines with respect to Australian biota and environmental conditions. A major objective of this study was to collate all known metal (including metalloid) toxicity data for aquatic biota in tropical Australia, as part of a broader study to test whether temperate North American metal toxicity data are applicable to the tropics of Australia.

The specific objectives of this study were to:

1 review available data on the toxicity of metals to aquatic biota in tropical Australia;

2 identify metals considered to be priority toxicants to aquatic biota in tropical Australia; and

3 employ previously developed toxicity testing protocols for two tropical freshwater species to obtain preliminary toxicity data for two priority metals.

From the literature review, it was concluded that insufficient metal toxicity data exist for Australian tropical species. Data were absent for a range of metals (eg Ag, As, Al, Cr, Hg, Ni, Sb and Se) listed in the current Australian water quality guidelines. Aluminium, Cd, Co, Cu, Ni, Mn, Pb, U, V and Zn were identified as priority metals of potential ecotoxicological concern in aquatic ecosystems of tropical Australia, largely as a consequence of mining activities, but also from urban impacts. Instead of testing the toxicity of the priority metals for which data do not currently exist (ie Al, Co, Ni and V), it was deemed more important to conduct further experimental work on Cu and U, in the context of elucidating the relatively high variability in the toxic response of these two metals. As a result, Cu and U were selected and toxicity tests conducted using two tropical freshwater species (green hydra (*Hydra viridissima*) and gudgeon fish (*Mogurnda mogurnda*)) from the Australian wet/dry tropics using test protocols designed to maximise the greatest sensitivity of metal response in the shortest period of time.

Population growth and survival were selected as the toxicological endpoints for *H. viridissima* and *M. mogurnda*, respectively. A four-parameter logistic regression model provided the best fit for the sigmoidal relationship between the selected responses of each organism and the measured total metal concentration. Using this regression model, the EC_{50} (median effect concentration) and an LC_{50} (concentration at 50% survival) were calculated for *H. viridissima* and *M. mogurnda*, respectively, for both Cu and U. The BEC_{10} (10% bounded effect concentration), an alternative statistical measure to the no-observed-effect-concentration (NOEC) and the MDEC (minimum detectable effect concentration), an alternative measure to the lowest-observed-effect-concentration (LOEC), were also calculated. A standardised synthetic test water (ie soft, slightly acidic, with a low buffering and complexation capacity), typical of sandy-braided streams throughout the Australian wet/dry tropics, was used for the experimental studies to maximise the toxic response to the organisms.

Results from the experimental work showed that *H. viridissima* were more sensitive to both Cu and U than were *M. mogurnda*. However, the difference in sensitivity was not equivalent for each metal. For example, the MDECs of Cu and U (as UO_2) for *H. viridissima* were 1.8 and 61 µg L^{-1}, respectively, whereas for *M. mogurnda* they were 13.4 and 1298 µg L^{-1},

respectively. *H. viridissima* was about eight times more sensitive to Cu than U, whereas *M. mogurnda* was about twenty times more sensitive. Once differences between the sublethal and lethal endpoints of the two organisms were corrected by statistical extrapolation, *H. viridissima* was approximately seven times more sensitive than *M. mogurnda* to U, but only about three times more sensitive to Cu. Both species were more sensitive to Cu than U. These results are generally consistent with those from previous studies when differences in key water quality variables, including water hardness, alkalinity, pH and dissolved organic carbon (DOC), are considered.

The current Australian water quality guidelines do not specify a protection level for U, or several other metals (eg Co, Mn and V), considered to be of potential toxicological concern in tropical Australian waters. Based on the results of this study, a guideline value of 5 $\mu g\ L^{-1}$ is recommended for total U in (tropical) Australia. Similarly, a guideline value of 1 $\mu g\ L^{-1}$ is recommended for total Cu in very soft waters (ie hardness < 10 $mg\ L^{-1}$ as $CaCO_3$). However, this guideline value approximates background (ie negligible anthropogenic disturbance) Cu concentrations measured in freshwater systems throughout tropical Australia.

The incorporation of key water quality variables, such as water hardness, alkalinity, pH and DOC (in the form of fulvic and humic acids), into further revisions of the Australian and New Zealand water quality guidelines could effectively address the issue of including bioavailable metal concentrations, instead of total concentrations. However, it is expected that only water hardness (which also incorporates alkalinity) will be used to quantitatively modify guideline values for selected metals (ie Cd, Cr(III), Cu, Ni, Pb and Zn) in the near future. It is recommended that further work should address the effects of pH and DOC (in the form of fulvic and humic acids) on the uptake and toxicity of Cu and U, as well as other priority metals of toxicological concern in tropical Australia. This information will potentially provide greater accuracy in determining the impact of metals in freshwater ecosystems, and thus, greater refine ecological risk assessments.

To provide a preliminary assessment of the suitability of the current Australian water quality guidelines for protecting aquatic life, a comparison of the toxicity of Cu and U to freshwater crustaceans and fish from North America and tropical Australia was performed. Copper and U were selected because they provided the most comprehensive toxicological dataset for tropical Australian species. The comparative study showed an overlap in the range of Cu and U toxicity values for both freshwater species of Crustacea and fish from tropical Australia and temperate North America, under conditions of comparable water chemistry. The level of confidence was greatest for Cu toxicity data on fish and least for U toxicity data on Crustacea. In general, few U toxicity data are available, particularly for North American species. Additional Cu toxicity data on tropical Australian species of crustaceans are required before reliable comparisons can be made with North American.

Based on several Australian toxicity studies, including this study, it was concluded that the Australian water quality guidelines, derived largely from North American toxicity data, are appropriate for Australian species and conditions when key water quality variables (eg temperature, water hardness and alkalinity) are considered. It is therefore proposed that North American toxicity data can be used to derive Australian water quality guidelines for protecting aquatic life in circumstances where toxicity data are either absent or scant for Australian aquatic life.

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Acknowledgments

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1 Introduction

The current Australian water quality guidelines for the protection of aquatic ecosystems (ANZECC 1992) are based predominantly on toxicological data derived from temperate North American biota. This philosophy was adopted because of a general paucity of toxicity data for Australian biota. Although local data are gradually increasing, and some chemicals are now generally well studied for a variety of Australian species, much more work is required. The fundamental question of whether overseas toxicity data are relevant to Australian species is frequently raised, given obvious differences in the climate, limnology and phylogeny of species. The lack of Australian toxicological data has constrained detailed comparisons with North American data. More specifically, the relevance of temperate North American toxicity data to tropical Australia (ie north of the Tropic of Capricorn—23.5°S) needs to be addressed, especially since the tropical zone comprises approximately 40% of the Australian continent (ASTEC 1993).

The development of a strategy for water quality management in tropical Australia is dependent on the quality of available toxicological data on Australian biota, and the capacity to predict the likely impacts of various chemicals on biota. To this end, the present work aims to review available data on the toxicity of metals (including metalloids) to aquatic biota in tropical Australia and, hence, identify where gaps occur. Having ascertained the gaps for metals that are considered to be of potential ecotoxicological concern in tropical Australia, there is a requirement to initiate studies to collect useful data for such metals. Beyond obtaining such data, there is a need to provide a model for predicting the potential impacts of specific metals on biota, based on key water quality variables, including water hardness (Ca + Mg)$_{,,}$ alkalinity, pH, salinity and dissolved organic carbon (DOC) (in the form of fulvic and humic acids). This is particularly relevant, since the Australian water quality guidelines are becoming increasingly used as part of the legislative framework for environmental protection, particularly in the field of environmental risk assessment. For example, the current Australian water quality guidelines (ANZECC 1992) do not specify a guideline for uranium (U) in aquatic ecosystems. However, a fundamental understanding of the potential impact of U on freshwater biota is relevant to assessing the risk of further U mining in Australia. Moreover, the requirement to perform ecologically relevant research in tropical Australia is consistent with the Australian Science and Technology Council report *Research and technology in tropical Australia* (ASTEC 1993).

In summary, the specific objectives of this study were to:

1 Review available data on the toxicity of metals to aquatic biota in tropical Australia;

2 Identify metals considered to be priority toxicants to aquatic biota in tropical Australia; and

3 Employ previously developed toxicity testing protocols for two tropical freshwater species to obtain preliminary toxicity data for two priority metals.

2 Literature review

2.1 Data selection

An extensive survey of the literature was performed for data on the toxicity of metals (including specific metalloids, ie As, Bi, Sb and Se) to Australian marine and freshwater biota from tropical Australia (comprising the wet, wet/dry and dry tropics and maritime area) (ASTEC 1993). It should be noted, however, that some biota have a natural distribution that

extends beyond the arbitrarily defined tropical boundary (see Section 1). In this study, metal toxicity data for some species were selected even though their natural tropical distribution was slightly exceeded. Most data were selected from refereed journal publications. Data were also obtained from government reports, book chapters or by personal communication with authors (usually where data were either not yet published or contained in commercial-in-confidence reports). All relevant data were considered up to, and including, January 1997. Only metals (including specific metalloids, ie As, Bi, Sb and Se) were considered; organo-metallics (eg tributyl tin, methyl mercury, methyl arsenate) were not included, as their mechanism of toxicity is not the same as metals (Waldock 1994). All toxicity data are reported as the individual metal (eg Cu), rather than the metal salt (eg $CuSO_4 \cdot 5H_2O$). The metals of organic salts (eg Pb-acetate) or experiments that used metal complexing buffers (eg phosphate) were not considered; such data underestimate the true metal toxicity (see Sections 2.2 and 5.2.2). Similarly, studies investigating the toxicological effects of metals in waste waters were not considered. Where toxicity endpoints (eg LC_{50}) were not reported, they were calculated (when possible) from the original data using appropriate statistical procedures (eg probit analysis).

All toxicity data were evaluated using the following quality criteria (after Emans et al 1993):

1 A distinct concentration-response relationship for each metal is obtained;

2 Such a relationship is derived using several increasing metal test concentrations, consisting of one control and at least three test concentrations;

3 Each control and metal concentration has at least one replicate;

4 The concentration of each metal should be measured throughout the experiment;

5 A defined toxicological endpoint (eg LC_{50}, EC_{50}) is obtained (or able to be calculated from the original data) for each metal, preferably with some measure of precision (where appropriate, ie 95% confidence limits for an LC_{50});

6 Biotic modifying factors relating to an organism are described (eg age, reproductive status, diet, life history etc);

7 Physico-chemical modifying parameters of the test water (eg temperature, salinity, pH, hardness, organic carbon) are measured;

8 The route (eg dissolved, particulate) and duration (eg continuous, pulsed) of metal exposure are defined;

9 The mode of metal exposure is described (eg static, flow-through); and

10 An organism is identified to at least the genus level, but preferably species.

Not all experiments were performed in accordance with all criteria. The original criteria were modified because some were found to be too stringent to be useful. Criteria 1–7 were considered the most important. An experiment may be classified as reliable if it satisfied criteria 1–7 and at least one of the less important criteria (ie criteria 8–10). All the surveyed data fell into this category. All data were arranged by broad taxonomic group (ie Crustacea, Mollusca etc).

2.2 Discussion

2.2.1 General observations

It was not the purpose of this work to provide a critical review of each selected study, rather to provide a summary of metal toxicity data on tropical aquatic biota, the conditions under

which the experiments were performed and a cursory commentary on trends shown from the data. The metal toxicity datasets for both freshwater and marine tropical biota are summarised in Appendixes A and B, respectively. From the data contained in Appendixes A and B, an overview of the types of metals and biota studied, as well as existing gaps in the literature, are shown in tables 1 and 2. These tables show the extent of metal toxicity data for Australian tropical freshwater and marine biota, respectively, in the context of the range of priority metals that have been reported for tropical aquatic biota outside Australia (ie Americas, Asia, Africa). The toxicity data contained in tables 1 and 2 were clarified and then summarised in tables 3 and 4. The measured toxicological endpoints (eg LC_{50}, EC_{50}) were derived from physiological and behavioural responses, individual and population growth, reproduction and survival across a range of life stages (eg embryos, juveniles, adults). Although some animal phyla were better represented than others (ie Chordata (fish) and Crustacea; table 3), and the toxicological endpoints varied considerably, all individual results were included to demonstrate the inherent variability in the data.

A comparison of metal toxicity data between plant and animal phyla is shown in tables 1–4. Tables 1 and 2 present metal toxicity data as summary statistics (mean, 95% confidence interval (CI), range and number of species studied). In instances where metal toxicity data followed a log-normal distribution, geometric mean values are reported. A comparison of the toxicity of some metals was initially precluded by differences in the duration of metal exposure and the selected toxicological endpoints (eg comparison of 96 h EC_{50} and 96 h LC_{50}). Such problems were overcome by using extrapolation models described by Hendriks (1995). Ratios between lethal versus sublethal endpoints, short-term versus long-term exposures and median versus no observed effect concentrations (NOECs) all seem to have an average value of 2–3 (Hendriks 1995). Thus, for comparative purposes, data were normalised to the most common toxicological endpoint (where applicable), namely the 96 h LC_{50}. For example, a 96 h EC_{50} for a particular species was multiplied by a factor of two (the conservative end of the range described by Hendriks (1995)) to achieve an estimate of the 96 h LC_{50}. Similarly, a 96 h NOEC was multiplied by a factor of four (ie two multiplied by two) to achieve an estimate of the 96 h LC_{50}. This process seemed rational given that there were no major differences in the ionic composition of the test waters used in the studies (see Section 2.2.2 and Appendixes A and B).

From the metal toxicity data given in tables 1–4, the following general trends are evident:

a) Only four freshwater phyla (ie Cnidaria, Mollusca, Crustacea and Chordata) comprising 24 species have been studied;

b) Only four marine phyla (ie Bacillariophyta (diatoms), Mollusca, Crustacea and Chordata) comprising 11 species have been studied;

c) The Chordata (fish) (15 species) and Crustacea (5 species) are the most frequently studied phyla for freshwater and marine biota, respectively;

d) Six metals have been studied for freshwater biota and seven for marine biota (with four metals in common ie Cd, Cu, Pb and Zn);

e) Uranium (18 species) and Cu (15 species) are the most frequently studied metals for freshwater biota;

f) Copper (7 species) and Zn (7 species) are the most frequently studied metals for marine biota;

g) Freshwater biota are generally more sensitive to Cd, Cu, Pb and Zn than marine biota;

h) Based on the metals tested, freshwater biota are most sensitive to Cu and least sensitive to Mn;

i) Similarly, marine biota are most sensitive to Hg and least sensitive to Ni and Pb;

j) The relative order of metal toxicity can vary considerably between freshwater and marine biota;

k) The Cnidaria, Crustacea and Mollusca are the more sensitive freshwater phyla to the metals studied; and

l) The Crustacea are generally the more sensitive marine phyla to the metals studied.

An anomalous result evident in table 1 is that the freshwater bivalve *Velesunio angasi* (Mollusca) is far less sensitive to Cu than freshwater species from other phyla. However, it has been well established that bivalves are able to reduce the exposure of their soft tissues from the aquatic medium by valve closure for extended periods of time (Markich 1995). Bivalves are not continuously exposed to a chemical during a normal 96 h LC_{50} test. Therefore, the 96 h LC_{50} for *V. angasi* is not comparable with other biota, such as crustaceans and fish, that are continuously exposed to a chemical. In effect, much longer metal exposures are required to attain an equivalent toxicity in bivalves. For example, Skidmore (1986) showed that the 33 d (792 h) LC_{50} for *V. angasi* exposed to Cu was 50 times lower than the 96 h LC_{50}. When valve closure is taken into account, the sensitivity of *V. angasi* to Cu is comparable with other phyla (Skidmore 1986). A further anomaly is that *V. angasi* is less sensitive to Cu than U and Mn (table 1), but this may be explained as follows. The 96 h LC_{50} concentrations for both U and Mn were extrapolated from a 96 h EC_{50} concentration that measured valve movement (ie closure) behaviour, and hence, normalised for an exposure period comparable with that of other phyla. In contrast, the measured 96 h LC_{50} for Cu greatly overestimates Cu toxicity because of valve closure.

2.2.2 Gaps in metal toxicity data

For several freshwater and marine plant and animal phyla, no toxicological data exist for tropical waters. There are no available metal toxicity data for freshwater plants (eg plankton and rooted emergent macrophytes), and only very limited data for marine plants (ie only the diatom *Nitzchia closterium*). A major problem identified with plant toxicity testing is that metal toxicity will invariably depend on the nature and concentration of chelators or complexing agents (eg EDTA, citrate, iron) in the assay medium (Stauber & Florence 1989). Complexing agents decrease metal toxicity by decreasing the free metal ion concentration (see Section 5.2.4). Stauber and Florence (1989) recommended the use of buffered synthetic soft water supplemented with nitrate and phosphate for freshwater plant assays, and unenriched seawater for marine plant assays, in an effort to standardise the assay medium, and thus, reduce the large variability inherent in toxicity studies with aquatic plants.

For the Chordata, only metal toxicity data for teleost fishes (Osteichthyes) are available for both fresh and marine waters. No data are available for Amphibia, Reptilia, Aves and Mammalia. Similarly, there are no available data for Uniramia (insects) or several other invertebrate phyla (eg Platyhelminthes, Nematoda, Annelida) with freshwater representatives. Although a general sublethal toxicity database for Mn and U is available for the freshwater bivalve *V. angasi*, there are no metal data for freshwater gastropod molluscs. Conversely, in the marine environment, there are no metal toxicity data for bivalves, with only limited Cu toxicity data for two congener species of gastropods.

Table 1 Summary of metal toxicity data for Australian tropical freshwater biota[a] (abstracted from Appendix A)

Metal	Parameter	Plants	Animals						
		Chlorophyta (Green algae)	Cnidaria	Mollusca	Annelida (Worms)	Crustacea	Uniramia (Insects)	Chordata	
								Osteichthyes (Fish)	Amphibia
Aluminium		–	–	–	–	–	–	–	–
Cadmium	Mean	–	–	–	–	–	–	57.6 (6470)	–
	95% CI							nc[b]	
	Range							13.4–160 (1500–18000)	
	No. species							3	
Chromium (VI)		–	–	–	–	–	–	–	–
Cobalt		–	–	–	–	–	–	–	–
Copper	Mean	–	0.13 (8.0)[c]	330 (21000)	–	0.72 (45.7)	–	1.86 (118)	–
	95% CI		nc[b]	nc[b]		nc[b]		0.87–2.86 (55.0–182)	
	Range		nc[b]	nc[b]		0.055–2.68 (3.5–170)		0.27–11.3 (23–720)	
	No. species		1	1		2		11	
Lead	Mean	–	–	–	–	2.41 (500)	–	25.2 (5220)	–
	95% CI					nc[b]		nc[b]	
	Range					nc[b]		0.87–154 (180–32000)	
	No. species					1		4	
Manganese	Mean	–	–	290 (15900)[d]	–	–	–	3440 (189000)	–
	95% CI			284–296 (15600–16260)				nc[b]	
	Range			277–306 (15200–16800)				186–>9100 (10200–>500000)	
	No. species			1				4	

Table 1 (cont'd)

Metal	Parameter	Plants	Animals						
		Chlorophyta (Green algae)	Cnidaria	Mollusca	Annelida (Worms)	Crustacea	Uniramia (Insects)	Chordata	
								Osteichthyes (Fish)	Amphibia
Mercury		–	–	–	–	–	–	–	–
Nickel		–	–	–	–	–	–	–	–
Silver		–	–	–	–	–	–	–	–
Uranium	Mean	–	2.77 (749)[e]	1.26 (340)[d]	–	1.34 (362)[e]	–	9.37 (2530)	–
	95% CI		nc[b]	0.72–2.68 (194–724)		nc[b]		6.59–12.2 (1780–3280)	
	Range		0.80–10.80 (244–2960)	0.48–5.00 (130–1350)		0.32–>21.1 (86–>5700)		3.07–>27.9 (830–7520)	
	No. species		2	1				10	
Vanadium		–	–	–	–	–	–	–	–
Zinc	Mean	–	–	–	–	6.58 (430)	–	46.3 (3030)	–
	95% CI					nc[b]		1–280 (65–18300)	
	Range					nc[b]		1–581 (65–38000)	
	No. species					1		7	

a Metal toxicity data (ie mean, 95% confidence interval and range) are expressed as a 96 h LC_{50} (or equivalent) in $\mu mol\ L^{-1}$ and $\mu g\ L^{-1}$ (in parentheses). Presentation of the metal toxicity as a micromolar concentration normalises for differences in molecular mass between metals. Uranium (U) concentration is expressed as uranyl (ie UO_2); this was derived by multiplying the concentration of U by 1.14. Australian data are shown in the context of the range of available toxicity data for tropical biota outside Australia.

b nc: not able to be calculated

c The 96 h EC_{50} was multiplied by a factor of two (Hendriks 1995) to derive a 96 h LC_{50}.

d The 96 h EC_{50} (Markich unpublished data) was multiplied by a factor of two (Hendriks 1995) to derive a 96 h LC_{50}.

e The 96 h LOEC was multiplied by a factor of four (Hendriks 1995) to derive a 96 h LC_{50}.

Table 2 Summary of metal toxicity data for Australian tropical marine biota[a] (abstracted from Appendix B)

Metal	Parameter	Plants	Animals					
		Bacillariophyta (Diatoms)	Cnidaria	Mollusca	Annelida (Worms)	Crustacea	Echinodermata (Sea urchins)	Chordata
Aluminium		–	–	–	–	–	–	–
Cadmium	Mean	–	–	–	–	3.90 (438)	–	136 (15300)
	95% CI					1.70–10.0 (191–1120)		59.1–275 (6640–30900)
	Range					1.33–16.5 (150–1850)		46.7–400 (5250–45000)
	No. species					4		2
Chromium (VI)	Mean	135 (7020)[b]	–	–	–	73 (3800)	–	–
	95% CI	nc[c]				nc[c]		
	Range	nc[c]				nc[c]		
	No. species	1				1		
Cobalt		–	–	–	–	–	–	–
Copper	Mean	–	0.410 (26.1)[d]	14.9 (947)	–	7.60 (483)	–	49.0 (3110)
	95% CI		nc[c]	nc[c]		nc[c]		33.1–71.9 (2100–4570)
	Range		nc[c]	12.1–18.4 (770–1170)		~0.50–96.0 (~16–6100)		33.1–94.4 (2100–6000)
	No. species		1	2		3		2
Lead	Mean	> 7.2 (> 1490)[e]	–	–	–	264 (54700)	–	660 (13700)
	95% CI	nc[c]				18.2–664 (3770–138000)		523–854 (108000–177000)
	Range	nc[c]				145–941 (30000–195000)		386–917 (80000–190000)
	No. species	1				1		2

Table 2 (cont'd)

Metal	Parameter	Plants	Animals					
		Bacillariophyta (Diatoms)	Cnidaria	Mollusca	Annelida (Worms)	Crustacea	Echinodermata (Sea urchins)	Chordata
Manganese		–	–	–	–	–	–	–
Mercury	Mean	–	–	–	–	0.429 (86.1)	–	2.31 (463)
	95% CI					0.060–1.14 (12.0–229)		1.71–3.01 (343–604)
	Range					0.150–1.45 (30.0–290)		1.65–3.29 (330–660)
	No. species					1		2
Nickel	Mean	> 34.0 (>2000)[e]	–	–	–	101 (5930)	–	818 (48000)
	95% CI	nc[c]				22.1–287 (1300–16800)		516–1234 (30300–72400)
	Range	nc[c]				22.1–358 (1300–21000)		511–1703 (30000–100000
	No. species	1				2		2
Vanadium		–	–	–	–	–	–	–
Zinc	Mean	6.0 (392)[b]	–	–	–	13.5 (883)	–	268 (17500)
	95% CI	nc[c]				nc[c]		223–320 (14600–20900)
	Range	nc[c]				5.66–101 (370–6600)		191–364 (12500–23800)
	No. species	1				4		2

a Metal toxicity data (ie mean, 95% confidence interval and range) are expressed as a 96 h LC_{50} (or equivalent) in µmol L^{-1} and µg L^{-1} (in parentheses) . Presentation of the metal toxicity as a micromolar concentration normalises for differences in molecular mass between metals. Australian data are shown in the context of the range of available toxicity data for tropical biota outside Australia.

b The 72 h EC_{50} was multiplied by a factor of two (Hendriks 1995) to derive a 96 h LC_{50}.

c nc: not able to be calculated

d The 4 h EC_{50} was multiplied by a factor of two (Hendriks 1995) to derive a 4 h LC_{50}. This value was then divided by two (Hendriks 1995) to estimate a 96 h LC_{50}.

e The 72 h LOEC was multiplied by a factor of four (Hendriks 1995) to derive a 96 h LC_{50}.

Table 3 Metal toxicity ranking for each phyla (based on data from tables 1 and 2)

Phyla	Freshwater	Species	Marine	Species
Plant				
Bacillariophyta	—	—	Zn > Cr	1
Animals				
Chordata (fish)	Cu > U[a] > Pb > Zn > Cd > Mn	15	Hg > Cu > Cd > Zn > Pb > Ni	2
Cnidaria	Cu > U[a]	2	Cu	1
Crustacea	Cu > U[a] > Pb > Zn	6	Hg > Cd > Cu > Zn > Cr> Ni > Pb	5
Mollusca	U[a] > Mn > Cu	1	Cu	2
Overall		**24**		**11**

a Uranium expressed as the uranyl ion (UO_2)

Table 4 Relative toxicity of individual metals between phyla (based on data from tables 1 and 2)

	Freshwater		Marine	
	Phyla[a]	Species	Phyla[a]	Species
Cadmium	Cho	3	Cru > Cho	6
Chromium(VI)	—	—	Cru > Bac	2
Copper	Cni > Cru > Cho > Mol	15	Cni > Cru > Mol > Cho	7
Lead	Cru > Cho	5	Cru > Cho; (Bac)	4
Manganese	Mol > Cho	5	—	—
Mercury	—	—	Cru > Cho	3
Nickel	—	—	Cru > Cho; (Bac)	5
Uranium	Mol > Cru > Cni > Cho	18	—	—
Zinc	Cru > Cho	8	Bac > Cru > Cho	7

a Bac: Bacillariophyta; Cho: Chordata; Cni: Cnidaria; Cru: Crustacea; Mol: Mollusca

The lack of toxicity data for specific phyla may be due to a variety of reasons. These may include (i) insufficient study of the general biology and ecology (including taxonomy) of species, (ii) species not being suitable for culture in the laboratory and (iii) lack of opportunity or motive to pursue the development of toxicity testing protocols for species whose biology and ecology is well established.

For freshwater biota, no toxicological data are available for Ag, Al, As, Cr, Co, Hg, Ni, Sb, Se and V (table 4), most of which are specified for the protection of aquatic ecosystems in the Australian water quality guidelines (ANZECC 1992). For marine biota, no toxicological data are available for Al, Co, Mn and V (table 4). Only scant metal toxicity data are available for Cd, Pb and Zn in freshwater systems (tables 1 & 4) and Cr and Hg in marine systems (tables 2 & 4).

Metal toxicity data for Australian tropical aquatic biota forms an important part of a larger metal toxicity database for tropical aquatic biota (Markich & Baird unpublished data). However, a larger variety of metals (including those listed in tables 1 and 2) and biota (ie

phyla shown in tables 1 and 2) have been studied in other tropical continents (ie Americas, Asia, Africa) relative to Australia. Gaps in Australian tropical data are obvious for several aquatic animal phyla, such as the Annelida, Uniramia, Echinodermata and Chordata (Amphibia), compared to data from other tropical continents. Only a very limited metal toxicity database exists for tropical aquatic plants outside Australia, where strong complexing agents have not been used in the assay medium (see Section 2.1.1). The only data available are for the toxicity of Cu to two species of green algae (Chlorophyta) from Papua New Guinea. Australia offers an exceptional U toxicity database for freshwater biota; one that is non-existent for other tropical continents.

The Australian freshwater studies reviewed were primarily conducted using either a natural or synthetic Magela Creek water (Alligator Rivers Region, Northern Territory, fig 1). This water is very soft and slightly acidic, with low levels of alkalinity, conductivity, turbidity and suspended solids (NT DTW 1983, Hart et al 1987, see also Appendix C.1). While the physico-chemistry of Magela Creek is typical of sandy braided streams in the wet/dry tropics during the wet season, it only indicates one of several general freshwater compositions in tropical Australia. Typically, Magela Creek waters are characterised by lower concentrations of dissolved salts than most other tropical Australian streams (B Noller pers comm). Experimental and/or field studies, where biota are exposed to metals in freshwaters with varying ionic composition, will complement existing metal toxicity data.

2.2.3 Variability in metal toxicity data

Despite the majority of tropical freshwater metal toxicity studies being conducted with Magela Creek water (or a synthetic analogue thereof), and marine metal toxicity studies being conducted in seawater, considerable variability exists between much of the metal toxicity data for a given species (where appropriate comparisons can be made). For example, the sensitivity (ie 48 h EC_{50}) of the freshwater bivalve, *V. angasi*, to U varies by about a factor of ten (117 to 1228 $\mu g\ L^{-1}$) (see Appendix A), while the sensitivity of the banana prawn, *Penaeus merguiensis*, to Zn varies by about a factor of twenty (370–6600 $\mu g\ L^{-1}$) (see Appendix B). Metal toxicity is known to be highly dependent on a variety of biotic (eg age/size) and water quality parameters (eg temperature, dissolved organic carbon (DOC), salinity, pH) (see review by Kong et al 1995). For this reason, it is essential to take into account these parameters in an attempt to reduce the inherent variability in the toxicity of a metal to an organism.

The effect of temperature can explain 94% of the variability in the response of the banana prawn to Zn (see Appendix B). Conversely, much of the variability in the toxicity of U and Cu to crustaceans and fish in natural Magela Creek water is difficult to explain, because key water quality parameters, such DOC and alkalinity, were not measured. This is particularly notable for DOC, which forms strong complexes with both U and Cu, and thus, may greatly ameliorate their toxicity to freshwater biota (Meador 1991, Markich et al 1996). Recent studies of U exposure to *V. angasi* (Markich et al 1996), which systematically varied the pH and DOC (in the form of a simulated fulvic acid; a major metal binding component of DOC) levels in synthetic Magela Creek water, were able to explain up to 96% of the variability in U response using these two parameters (see also Section 5.2.4). Further studies of this type are recommended to reduce variability, and thus, provide predictive models that enhance interpretation of metal toxicity results.

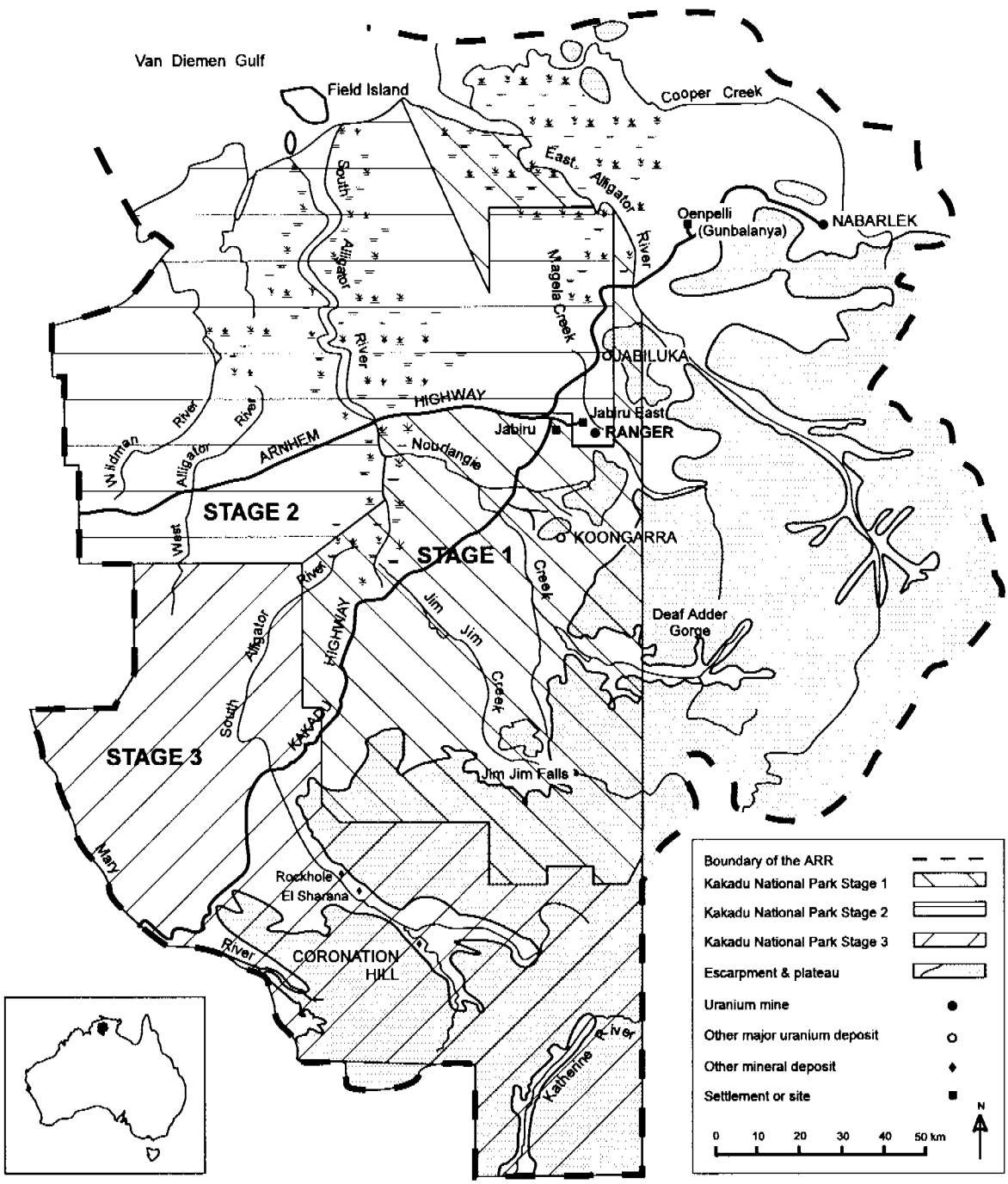

Figure 1 Alligator Rivers Region

Other factors, that are inherent to the experimental design and data analysis, may also influence the variability of a toxic response to a metal. Many of the earlier studies (eg Giles 1974) relied on nominal metal concentrations with static exposures to derive metal toxicity values. With static exposures, the concentrations of metals invariably decline over the period of exposure due to adsorption (to the test containers) and precipitation, particularly where pH and alkalinity increase in the water over the test period. Hence, test organisms are not exposed to a constant concentration(s) of a metal, leading to differences between nominal and measured metal concentrations that may be considerable.

Best practice advocates the use of static-renewal or flow-through exposure designs where the ionic composition and, hence, the metals of interest, are measured using appropriate analytical techniques. Furthermore, the choice of statistical methods for data reduction introduces some variation in the calculated toxicological endpoint. Such variability is expected to be greatest for studies employing graphical interpolation techniques (as opposed to routine computer calculations), especially for metals with high toxicity (ie differences are maximised between smaller toxicity values). Chapman et al (1996) cites a case where toxicity tests in different laboratories generated NOECs that were different by up to a factor of nine because slightly different experimental and statistical methods were used.

3 Metals of toxicological concern to aquatic biota in tropical Australia

Aluminium, Cd, Co, Cu, Ni, Mn, Pb, U, V and Zn have been identified as priority metals of potential ecotoxicological concern in aquatic ecosystems of tropical Australia, largely as a consequence of mining activities, but also from urban impacts. This list of metals was derived from consultation with a variety of government agencies (eg Northern Territory Department of Mines and Energy; Queensland Department of Primary Industries and Fisheries) and non-government organisations (eg Energy Resources of Australia) with interests in tropical Australia. For several of these metals (ie Al, Co, Ni and V), there are no toxicological data for Australian tropical aquatic biota (see Section 2.2.2).

Instead of testing the toxicity of Al, Co, Ni and V in this study, it was deemed more important to conduct further experimental work on Cu and U, in the context of elucidating the relatively high variability in the toxic response of these two metals in natural Magela Creek water (see Appendix A). Copper and U were selected because they provide the most comprehensive database for the toxicity of metals to tropical freshwater biota. Thus, the paradox exists that even when satisfactory toxicological data for a particular metal are available, the predictive capability of the data are reasonably poor. This study will test the toxicity of Cu and U using a standard synthetic water that closely resembles the inorganic composition of Magela Creek during the wet season. The wet season is the time when the vast majority of streams flow in tropical Australia and when mine discharges and urban runoff usually occur.

The incorporation of a synthetic standard water chemistry into established toxicity testing protocols would provide a baseline (a) from which a large range of different water quality parameters could be calibrated and assessed against natural water, and (b) for providing a high risk scenario, with respect to assessing the toxicity of metals. It is envisaged that such an approach will help to clarify previous data for these two metals so that a stronger predictive capability for assessing ecological risk can be gained from the joint data. This process could then be extended to other priority metals of toxicological concern to aquatic biota in tropical Australia.

4 Test species

4.1 Existing test protocols

Existing test protocols (Hyne et al 1996) for two organisms, green hydra (*Hydra viridissima*; fig 2) and the purple-spotted gudgeon (*Mogurnda mogurnda*; fig 3) were modified (see Section 4.2) and then tested with Cu and U using a synthetic Magela Creek water. Details of the protocols, including the synthetic water chemistry, are described in Appendix C. A detailed description of the rationale and process used to select suitable freshwater test species in tropical Australia, and appropriate toxicological endpoints, has been previously reported (Holdway et al 1988, Hyne et al 1996). Two additional test protocols, one using the water flea *Moinodaphnia macleayi* and the other using the green alga, *Chlorella* sp. (new species), are currently being developed for use in synthetic Magela Creek water. The freshwater bivalve *V. angasi* is also a useful test species that has already undergone extensive development.

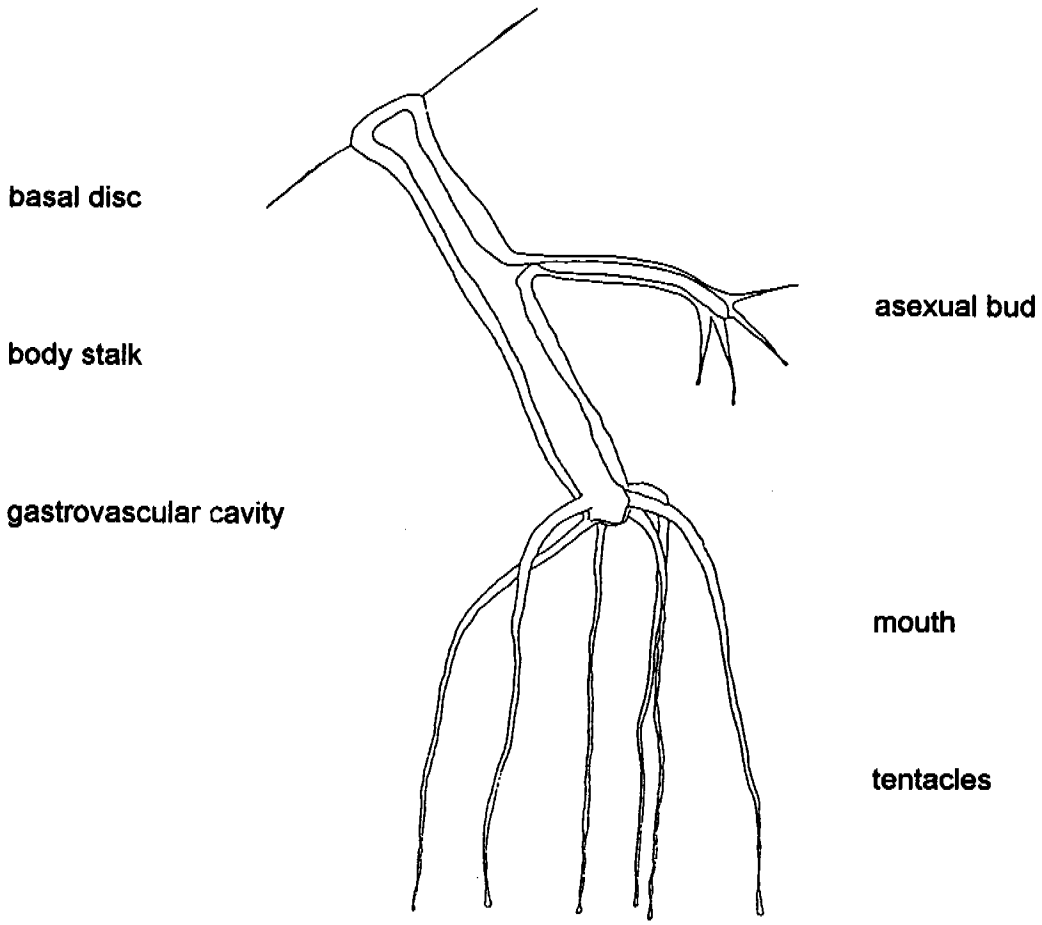

Figure 2 Diagram of *H. viridissima*

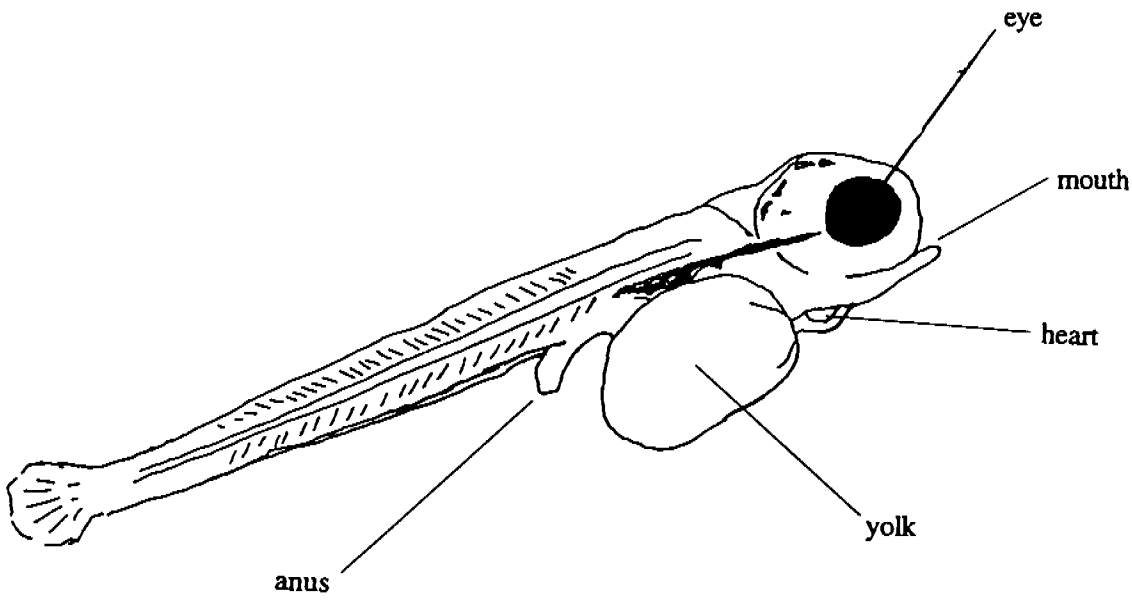

Figure 3 Diagram of the sac-fry larvae of *M. mogumda*

4.2 Improvements to existing test protocols

Several improvements to existing test protocols (Hyne et al 1996) were made as part of this work, and are listed as follows:

a) Synthetic water was successfully incorporated into the test protocols for green hydra and the purple-spotted gudgeon (Appendix C);

b) The hydra test was shortened from 6 to 4 days without compromising the sensitivity and statistical significance of the selected toxicological endpoints (Appendix C);

c) The gudgeon test was simplified (Appendix C). The previous protocol included both an embryo and sac-fry component to the test. Only the sac-fry component was retained. Embryos were often subject to (a) damage, when removed from their substrate, (b) fungal contamination and (c) non-fertilisation, resulting in non-viable embryos. Without an embryonic component to the gudgeon test, the manipulation of animals is made easier at the beginning of the sac-fry test, with less potential for damage. Additionally, healthy animals (ie animals without overt signs of disease or deformity) can be selected. This process was not possible at the embryo stage. Furthermore, the sac-fry stage is known to be a more sensitive life stage (Rippon & Hyne 1992); and

d) The test protocols for green hydra and the purple-spotted gudgeon were successfully performed at 27°C (30°C was specified previously) without compromising developmental and reproductive rates or sensitivity in the selected toxicological endpoints.

Overall, both test protocols were optimised to provide the greatest sensitivity of chemical response in the shortest period of time.

5 Toxicity of Cu and U to *H. viridissima* and *M. mogurnda*

5.1 Methodology

Using the test protocols described in Appendix C, the comparative toxicity of Cu and U to *H. viridissima* and *M. mogurnda* was investigated. From the results of range-finding studies for each metal, definitive toxicity experiments were conducted. This included a complete concentration-response relationship for *H. viridissima,* but only a partial concentration-response relationship for *M. mogurnda* (consistent with the objective of evaluating the no- and lowest-observed effect concentrations, see Appendix C.1.1).

5.1.1 Physico-chemical analysis

Sub-samples of the synthetic test water were analysed using a combination of analytical techniques. The concentrations of Na, K, Ca, Mg, NH_4, Cl, SO_4 and NO_3 were measured on filtered (0.45 µm; Millipore), unacidified samples by high performance liquid chromatography (leGras 1993). Concentrations of Al, Fe and Mn were measured by inductively coupled plasma atomic emission spectrometry (Spectroflame), while Cu, Pb, U and Zn were measured by inductively coupled plasma mass spectrometry (Fisons VG PQ2). Trace metal determinations were performed on acidified (pH <2) samples. A multi-ion calibration standard and a reagent blank were analysed with every 10 samples to monitor signal drift. In every instance, and for all ions, the signal changed by less than 8%, but typically 3–5%. Where inductively coupled plasma mass spectrometry was used, gallium, indium and rhenium were employed as internal standards to correct for non-spectral interferences.

Alkalinity was determined using APHA et al (1995) standard method 2320B.*4d*. Bicarbonate concentrations were determined nomographically from alkalinity measurements using standard method 4500-CO_2 (APHA et al 1995). Organic carbon was measured using an OIC Model 700 Total Carbon Analyser (ie persulphate oxidation method). pH and conductivity were measured using an Alpha pH/conductivity meter. An epoxy-body combination pH electrode (Sensorex) was calibrated daily with standard buffer solutions (BDH). A platinum/glass conductivity cell (EDT) was calibrated daily with standard KCl solutions. Dissolved oxygen (DO) concentration was measured using a polarographic electrode coupled to an Activon Model 401 oxygen meter. pH, DO and conductivity were measured daily; pH was adjusted to the nominal value (ie pH 6.00 ± 0.15) when required.

The results of chemical analyses showed that the mean concentrations of all ions in the test waters (measured at the start (t=0 h) and end (t=96 h) of each test) were usually within 7%, but always <15%, of their nominal concentrations (see Section C.1.7). Measured, not nominal, concentrations of Cu and U were used to assess the concentration-response relationships for each organism. Uranium occurs in the environment in several oxidation states; however, the hexavalent (UO_2^{2+}; uranyl ion) state predominates in oxidised waters, and hence, has been used to represent U in this study.

Quality assurance procedures were followed for all Cu and U analyses. A duplicate sample and standard reference materials (National Institute of Standards and Technology, Trace elements in water 1643c and National Research Council of Canada, Riverine water for trace metals SLRS-2) were analysed with each batch of ten samples to measure method precision and accuracy, respectively. The mean concentrations of Cu and U in the standard reference materials were within their certified concentration ranges. The percentage coefficient of variation (% C.V.) for duplicate sample analyses averaged 7% for both metals.

5.1.2 Geochemical speciation modelling of Cu and U

The speciation of a metal in solution governs it bioavailability (ie the ability to interact with a biological cell membrane), and hence, its toxicity to aquatic biota (see Section 5.2.4). The thermodynamic geochemical speciation code HARPHRQ (Brown et al 1991) was employed to predict the speciation of Cu and U in the test water. The input parameters for HARPHRQ were based on physico-chemical data measured from the test waters. Equilibrium constants for the inorganic U species used in the geochemical simulations were derived primarily from the Nuclear Energy Agency (NEA) critical review series (Grenthe et al 1992, 1995), but also from original research publications (Choppin & Mathur 1991, Palmer & Nguyen 1995), where data were deemed to be consistent. Equilibrium constants for the inorganic Cu species, as well as other inorganic metal species in the test water, were derived primarily from critical literature compilations and/or reviews (eg Nordstrom et al 1990, Smith et al 1995).

5.1.3 Data analysis

The 10% bounded effect concentration (BEC_{10}), an alternative statistical measure to the no-observed effect concentration (NOEC), was estimated using the approach described by Hoekstra and van Ewijk (1993). The minimum detectable effect concentration (MDEC), an alternative measure to the lowest-observed effect concentration (LOEC), was estimated using the approach described by Ahsanullah and Williams (1991). A four-parameter logistic regression model (Guardabasso et al 1987, Seefeldt et al 1995) provided the best fit for the sigmoidal relationship between the selected responses of each organism and the measured total metal concentration. The concentration-response relationships are described by the following equation:

$$Y = \frac{a - d}{1 + (x / c)^{b+d}}$$

where Y is the response: x, the arithmetic metal concentration: a, the minimum calculated response: d, the maximum calculated response: c, the EC_{50}, ie the concentration resulting in a response halfway between a and d: and b is the 'slope' around the EC_{50}.

Using this logistic regression model, the EC_{50} (and its 95% confidence interval) was calculated for *H. viridissima* with both Cu and U. Model parameter estimates were derived by the method of maximum likelihood with a binomial probability distribution. Model adequacy was evaluated using a χ^2 goodness of fit test (Helsel & Hirsch 1992) and confirmed in all cases. By combining the definitive response data with the range-finding data, an LC_{50} was also calculated for *M. mogurnda* with both metals using the logistic regression.

Plots generated from the concentration-response data given in Appendix D showed that the regression relationships for each individual test-run of a given metal-organism exposure (eg five Cu test-runs were performed with hydra; Appendix D) could be adequately described by linear models for the purpose of ANCOVA (analysis of covariance) (plots not provided here). From ANCOVA, it was shown that the regression slopes for each test-run of a given metal-organism exposure did not significantly ($P \leq 0.05$) differ when the composition of the test waters remained constant. These results validated the mean, pooled data for derivation of BEC_{10}, MDEC and $E(L)C_{50}$ values, and parameter estimates for the logistic regression models.

5.2 Results and discussion

5.2.1 Toxicity of Cu and U to *H. viridissima* and *M. mogurnda*: Comparison with previous toxicity studies

Plots of the concentration-response relationships for *H. viridissima* and *M. mogurnda* are shown in figures 4–7. Summary data for each concentration-response relationship are given in Appendix E. Raw data for each test-run of a given metal-organism exposure are provided in Appendix D.

The toxicity endpoints calculated for each metal are shown in table 5. The results, based on the BEC_{10} and MDEC values, show that *H. viridissima* is more sensitive than *M. mogurnda* to both Cu and U (hereafter expressed as uranyl, UO_2). However, the difference in sensitivity is not equivalent for each metal. *H. viridissima* is approximately twenty times more sensitive than *M. mogurnda* to U, and only about seven times more sensitive to Cu. Note that the EC_{50} values of U and Cu for *H. viridissima* are not directly comparable with the LC_{50} values for *M. mogurnda*. The former is a measure of a sublethal endpoint (ie population growth) and is generally considered to be more sensitive than lethality (Hendriks 1995). However, a reasonable comparison can be made if the LC_{50} values for *M. mogurnda* are divided by an extrapolation factor of two (Hendriks 1995, see also Section 2.2.1). This results in an estimated EC_{50} value of 784 µg L^{-1} (or 2.90 µmol L^{-1}) for U and 11.3 µg L^{-1} (or 0.18 µmol L^{-1}) for Cu. Based on this endpoint, *H. viridissima* is approximately seven times more sensitive to U, and only about three times more sensitive to Cu, than *M. mogurnda*.

Table 5 Toxicity of U and Cu to *H. viridissima* and *M. mogurnda*[a]

Metal	WQG[b]	*H. viridissima*			*M. mogurnda*		
		BEC_{10}	MDEC	EC_{50} (95% CI)	BEC_{10}	MDEC	EC_{50} (95% CI)
U	ngv[c]	56	61	108 (102–114)	1265	1298	1568 (1511–1625)
		[0.21]	[0.23]	[0.40] [0.38–0.42]	[4.68]	[4.81]	[5.81] [5.60–6.02]
Cu	1–5[d]	1.6	1.8	4.0 (3.75–4.25)	12.2	13.4	22.7 (21.5–23.9)
		[0.025]	[0.028]	[0.063] [0.059–0.067]	[0.19]	[0.21]	[0.36] [0.34–0.38]

a Toxicity endpoints (ie BEC_{10}, MDEC and EC_{50} at 96 h) are expressed as µg L^{-1} and µmol L^{-1} [in brackets] (see Section 5.1.3). Uranium is expressed as uranyl (UO_2) (ie U x 1.14).

b WQG: Water quality guideline for the protection of freshwater life (ANZECC 1992).

c ngv: no guideline value.

d Dependent on water hardness (although an algorithm is not reported in the 1992 Australian water quality guidelines).

Both species are more sensitive to Cu than U. If the toxicity of these two metals is compared on a molar basis (ie to account for differences in molecular mass), U is approximately eight times less toxic than Cu for *H. viridissima* and twenty times less toxic for *M. mogurnda*. Recent investigations (Markich unpublished data) on the valve movement behaviour (ie duration of valve gape) of the freshwater bivalve *V. angasi*, using the same water quality conditions, indicate that this species is approximately three times less sensitive to U than *H. viridissima*, but about two times more sensitive than *M. mogurnda* (based on an equivalent comparison using 96 h EC_{50} values).

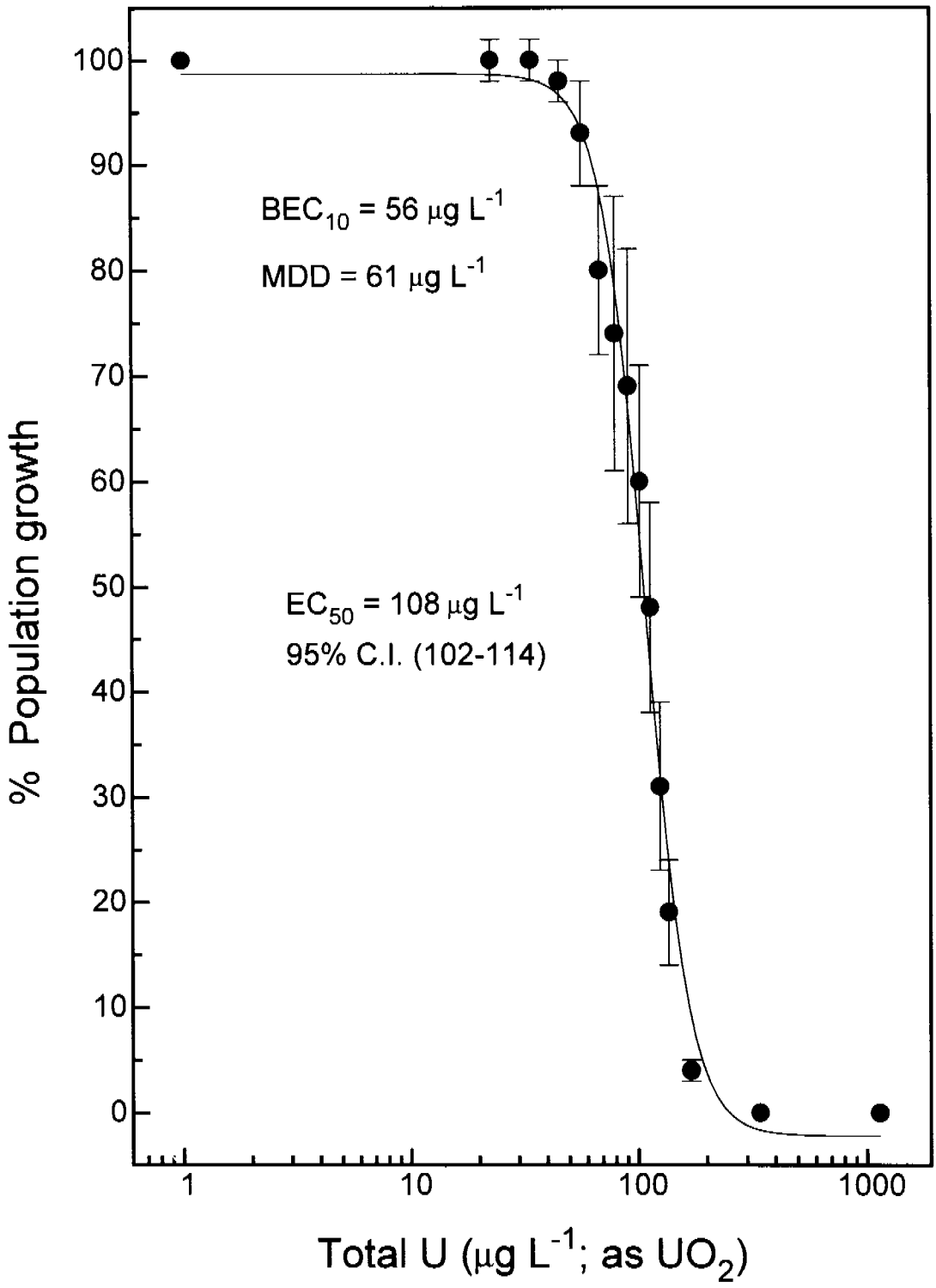

Figure 4 Percentage population growth of *H. viridissima* plotted against total U concentration. Each plotted point represents the mean and 95% confidence interval. The toxicological endpoints (ie BEC_{10}, MDEC and EC_{50} at 96 h) are described in Section 5.1.3.

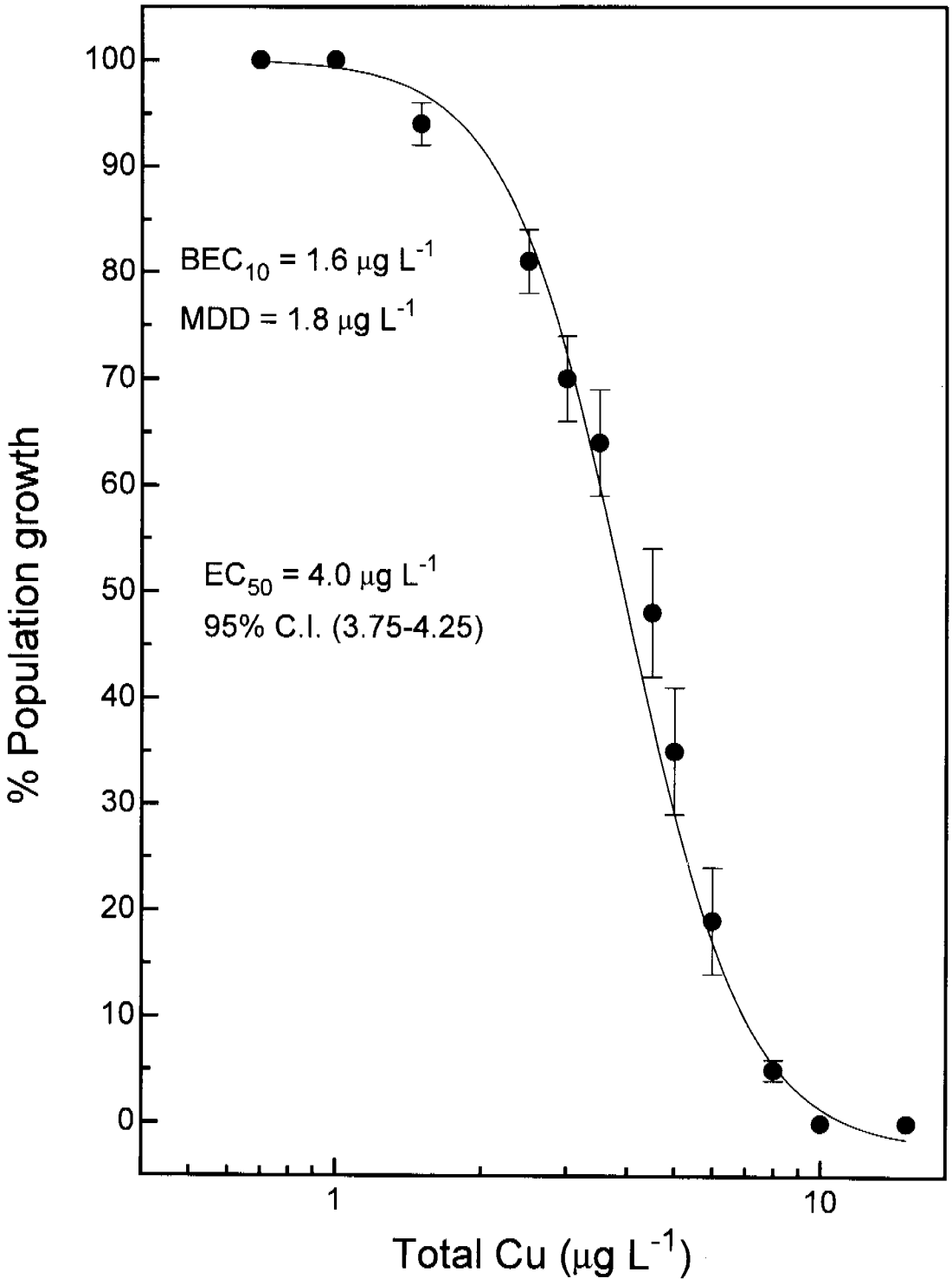

Figure 5 Percentage population growth of *H. viridissima* plotted against total Cu concentration. Each plotted point represents the mean and 95% confidence interval. The toxicological endpoints (ie BEC_{10}, MDEC and EC_{50} at 96 h) are described in Section 5.1.3.

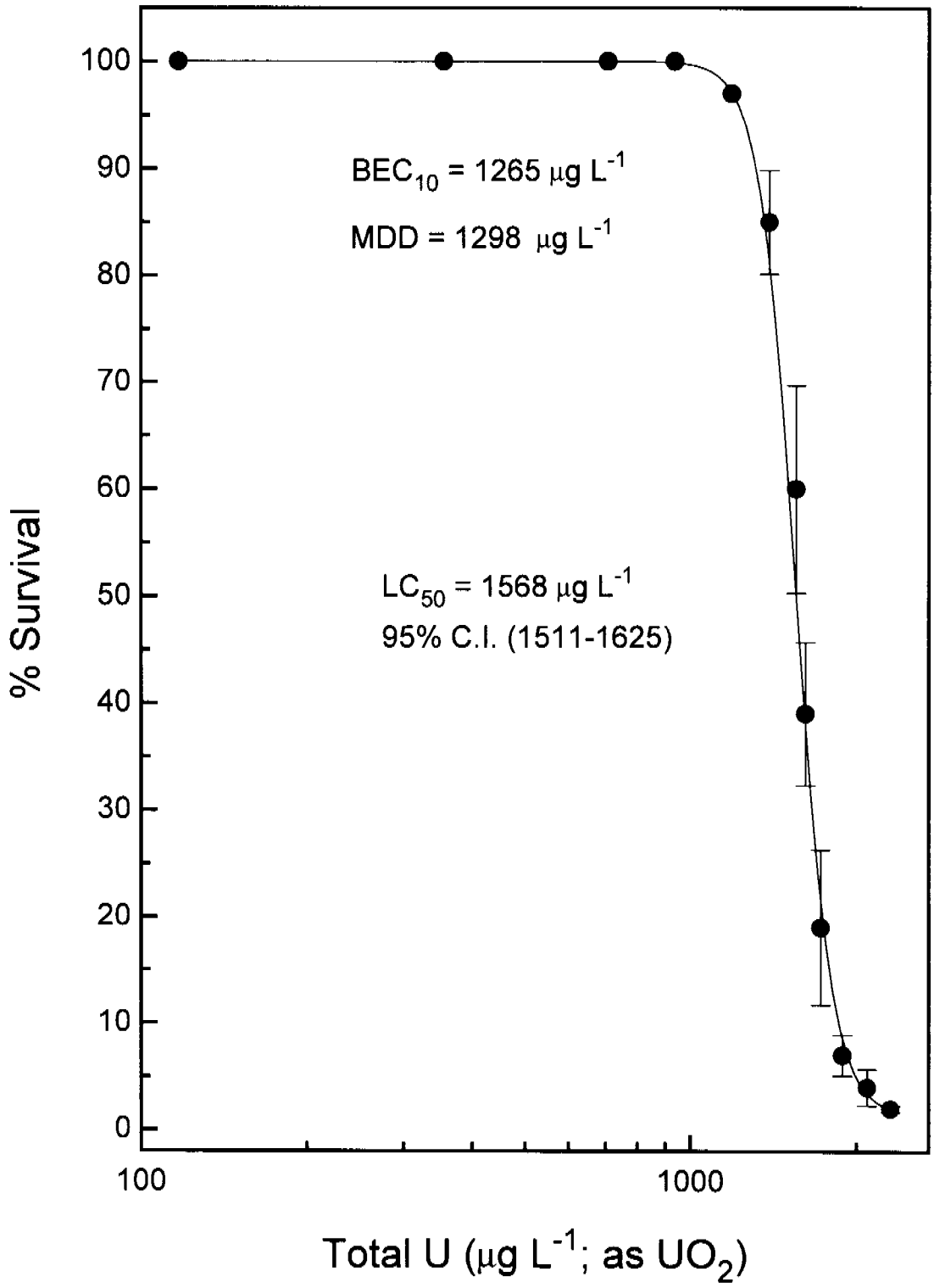

Figure 6 Percentage survival of *M. mogumda* plotted against total U concentration. Each plotted point represents the mean and 95% confidence interval. The toxicological endpoints (ie BEC_{10}, MDEC and LC_{50} at 96 h) are described in Section 5.1.3.

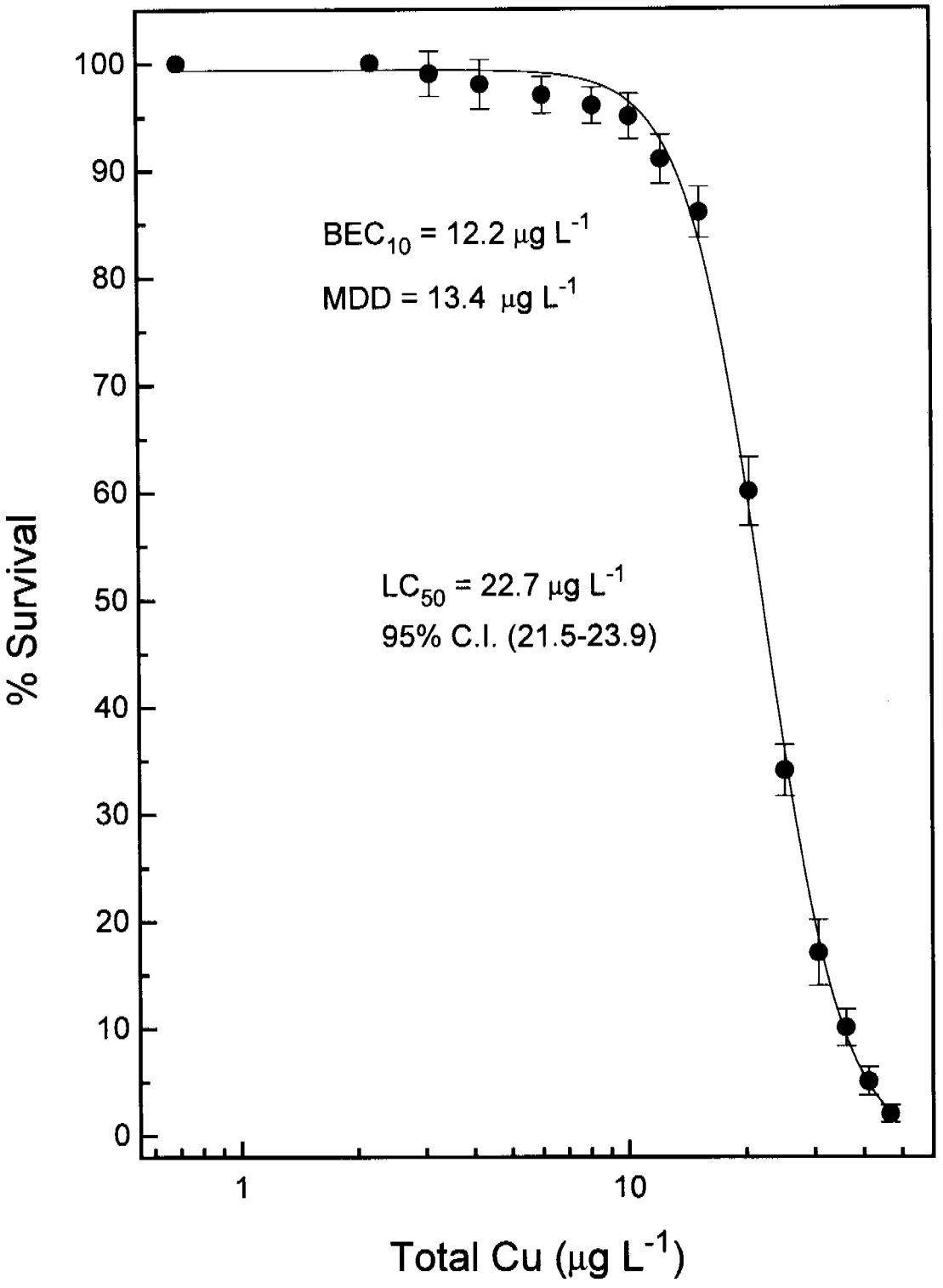

Figure 7 Percentage survival of *M. mogumda* plotted against total Cu concentration. Each plotted point represents the mean and 95% confidence interval. The toxicological endpoints (ie BEC_{10}, MDEC and LC_{50} at 96 h) are described in Section 5.1.3.

The toxicity of U to *H. viridissima* reported here is about a factor of three times greater than that reported in a previous study which exposed this species to U in natural Magela Creek (ie Buffalo Billabong) water (see Appendix A). Although the ionic composition of Buffalo Billabong water was very similar to the synthetic water used in this work, the concentration of DOC in the billabong water was not reported, and the pH was 0.5 units greater (ie 6.5) in the natural water. The observed difference in U toxicity most probably stems from a reduction in the bioavailable fraction in the natural water as a result of uranyl-organic complexation (see Section 5.2.4). *H. viridissima* has been shown to be about a factor of four to five times more sensitive to U than the pink hydra, *Hydra vulgaris* (Appendix A). No other studies have reported the toxicity of U or Cu to tropical species of hydra, or other Australian freshwater representatives of Cnidaria. An important feature evident in fig 5 is the relatively small difference in the total Cu concentration between the no-effect concentration (BEC_{10} = 1.6 µg L^{-1}) and the concentration responsible for a 50% decline in population growth (EC_{50} = 4.0 µg L^{-1}) for *H. viridissima*. This small toxicity 'window' may have important implications for the protection of this species to acute Cu exposures. Similar trends are also evident for U.

The toxicity of U measured here for *M. mogurnda* is very similar to previously reported results (Bywater et al 1991, Holdway 1992) for this species in natural Magela Creek water at a similar life stage (ie sac-fry & larvae). However, it was expected that *M. mogurnda* would be more sensitive to U in the synthetic water, as shown for *H. viridissima*, because it had a slightly lower pH (ie ~0.5 units lower) and a lack of DOC compared with natural water (see Appendix A). These differences in water chemistry are known to enhance the toxicity of U to biota (see Section 5.2.4). A closer examination of variability in water quality and biotic modifying factors may be required to help elucidate this result.

Longer-term lethal exposures (ie 14 d) of U to sac-fry of *M. mogurnda* have also been reported by Holdway (1992). Holdway found that the sensitivity of *M. mogurnda* to U does not necessarily increase with increasing exposure time, at least over 14 d (Appendix A). Bywater et al (1991) compared the relative sensitivity of U to five species of fish from Magela Creek at various life stages (see Appendix A). *M. mogurnda* was shown to be the second most sensitive species, being only less sensitive than the blue eye (*Pseudomugil tenellus*), with the Mariana's hardyhead (*Craterocephalus marianae*), black-striped rainbowfish (*Melanotaenia nigrans*) and chequered rainbowfish (*Melanotaenia splendida*) all progressively more tolerant to U.

The toxicity of Cu to the sac-fry of *M. mogurnda* reported here is approximately twice that reported by Rippon and Hyne (1992) who used natural Magela Creek (Buffalo Billabong) water (Appendix A). In contrast to the results described above for the toxicity of U to *M. mogurnda*, the observed differences in Cu toxicity between the two studies most probably stem from a reduction in the bioavailable Cu concentration in the natural water as a result of Cu-organic complexation (see Section 5.2.4). Rippon and Hyne (1992) did not measure the DOC concentration in their test water. In the absence of a single study comparison of the toxicity of Cu to a range of fish that include *M. mogurnda*, the combined results of several studies covering 11 species (see Appendix A) indicate that *M. mogurnda* is among the more sensitive species of fish to Cu. This is consistent with the findings of Bywater et al (1991) for U toxicity. As noted above for *H. viridissima* exposed to Cu in this study, there is only a relatively small difference between the concentration of Cu that results in no mortality (BEC_{10} = 12.2 µg L^{-1}) and that which produces 50% mortality (LC_{50} = 22.7 µg L^{-1}).

5.2.2 Advantages of using concentration-response statistics to derive toxicity values

Ecological risk assessments of chemicals are usually concerned with determining whether levels in the environment could, or are exceeding, predicted no-effect concentrations (Environment Canada 1996). Thus, the goal of most toxicity tests is to estimate no or low toxic effects concentrations, which may then be used as input into risk assessments or the development of environmental (water) quality guidelines for risk management purposes. The statistical approach most commonly used to estimate no or low toxic effects is hypothesis testing, in which treatment responses are compared with a control response to test the null hypothesis that they are the same. If the null hypothesis is rejected, a multiple comparison test (eg Newman-Keuls, Dunnetts, Scheffé) is used to generate a NOEC and a LOEC.

The use of NOECs and LOECs as the basis for estimating "no effects" concentrations has been widely criticised (Stephan & Rogers 1985, Bruce & Versteeg 1992, Hoekstra & van Ewijk, 1993, Suter 1996, Chapman et al 1996) for a variety of reasons, including:

a) hypothesis testing procedures clearly state the α value but generally leave the β value unconstrained, meaning that the typical test will err on the side of stating there is no toxicity present even when it is (a type II error) (Peterman & M'Gonigle 1992, Power et al 1995);

b) the NOEC and LOEC are always test concentrations and do not innately correspond to biologically relevant thresholds or specified effects concentrations;

c) poor experimental design (eg small sample size, improper spacing of treatment concentrations, large intra-treatment variance) can mistakenly indicate that the substance is less toxic than it really is (Stephan & Rogers 1985, Barnthouse et al 1987); and

d) most of the information in the concentration-response curve (eg slope, confidence limits) is lost and, thus, the investigator has no means of evaluating the test results and cannot, for example, use the results to estimate risks of varying severity.

An alternative for estimating low toxic effects is the regression-based approach. This approach involves fitting a regression model equation (eg logistic, probit) to toxicity test results to estimate the concentration-response function and then interpolating or extrapolating to the effect concentration of interest (eg EC_{25}). The analysis may be done by means of a non-linear regression or a weighted linear regression on transformed data (Nyholm et al 1992). Some of the major advantages of the concentration-response approach over hypothesis testing for estimating low toxic effects include:

a) it is a well-defined procedure for interpolation of effects to untested concentrations;

b) test statistics can determine whether model fit is adequate and whether the assumptions of the analysis have been met, thus precluding the use of poor quality information or inappropriate models; and

c) all the information in the concentration-response curve may be used in the analysis (eg decisions on the benchmark concentration and the acceptable environmental concentration can be based on biological effects) (Stephan & Rogers 1985, Bruce & Versteeg 1992, Moore & Caux 1997).

5.2.3 Derivation of Cu and U guideline values to protect Australian freshwater life

The 1992 Australian water quality guideline for total Cu ranges from 2 to 5 $\mu g\ L^{-1}$ (depending on water hardness) to protect freshwater ecosystems (ANZECC 1992). Unfortunately, no quantitative formulation is provided in the guidelines that specifies a particular Cu concentration for a given water hardness. The Australian water quality guidelines for the

protection of aquatic life are currently being revised and should be completed by 1998. The revised guidelines will include algorithms that can be used to derive a guideline value for a selection of metals (ie Cd, Cr(III), Cu, Ni, Pb and Zn) whose toxicity is modified by water hardness (Markich et al 1997). Given that the hardness of Magela Creek water is extremely low (typically 3–10 mg L^{-1} as $CaCO_3$), a total Cu concentration of 1 µg L^{-1} would not be an unreasonable guideline value based on the 1992 water quality guidelines. In this study, *H. viridissima* detected Cu at 1.8 µg L^{-1} and had an EC_{50} of 4 µg L^{-1} (table 5). Hence, *H. viridissima* shows an adverse response to Cu at concentrations similar to those listed in the 1992 guidelines. *M. mogurnda* detected Cu at 12 µg L^{-1} (table 5); 12 times greater than the proposed Cu guideline for Magela Creek.

Although the background (ie negligible anthropogenic disturbance) concentration of Cu in the the surface waters of the Magela Creek, and many other sandy-braided streams in the wet/dry tropics, is typically 0.70 µg L^{-1} (Hart et al 1987), streams draining mineralised (eg base metal) deposits may naturally reach 5 µg L^{-1}. Hence, there would appear to be very little scope to alter the proposed guideline values of Cu in such freshwater systems, particularly when errors associated with (a) analytical techniques for measuring total Cu in water and (b) data reduction from toxicity tests are considered. In situations where the measured background metal concentration approximates the total metal guideline concentration, it will become increasingly important that state-of-the-art, ultraclean, sampling, handling, processing and analytical techniques (see Ahlers et al 1990, Nriagu et al 1996) be adopted by water scientists to minimise contamination and, hence, provide sensitive background metal concentrations for aquatic systems that can serve as benchmarks to assess environmental contamination.

Copper is known to be one of the more toxic metals to freshwater biota (see reviews by Harrison & Bishop 1984, Nor 1987, Markich et al 1997). It should be noted that the synthetic water used for this study lacked any organic chelating agents (ie DOC) and represented a high risk scenario. Like U, Cu forms strong complexes with DOC, and thus, its toxicity to freshwater biota can be substantially ameliorated (Meador 1991, Erickson et al 1996). However, only very low concentrations of DOC (1–3 mg L^{-1}) occur in Magela Creek during normal flow conditions (C leGras pers comm). Further work is required to assess the influence of DOC and other key water quality parameters, such as water hardness, alkalinity and pH, on the bioavailability and, hence, toxicity of Cu to Australian tropical freshwater biota, with the goal of further improving environmental protection. Additionally, further work on the sublethal effects on fish are required over longer times scales to ascertain the sensitivity of Cu.

A water quality guideline for U does not currently exist for the protection of freshwater (or marine) ecosystems in Australia. This is a noticeable shortcoming given Australia's historical background, as well as current and future interests, in U mining, particularly in the tropics. The Pine Creek Inlier (66 000 km^2, situated between Darwin and Katherine, NT) is Australia's largest and richest uranium province, containing the Alligator Rivers, Rum Jungle and South Alligator River Valley uranium fields (Needham & De Ross 1990). Of the freshwater streams draining the Pine Creek Inlier, the Magela Creek (and its catchment) has been the most widely studied because of its proximity to the Ranger uranium mine within Kakadu National Park (fig 1). Thus, it was considered appropriate to use Magela Creek water as the benchmark to derive a national water quality guideline for U, until additional toxicity data become available for U in other tropical freshwater systems. An interim guideline of 5 µg U L^{-1} is currently being used for the protection of freshwater ecosystems in the Magela Creek by the Northern Territory Department of Mines and Energy (NT DME 1982).

It is widely recognised that data derived from at least four different single species toxicity tests, where each test species is representative of different trophic levels (ie producer, herbivore or consumer), are required to adequately formulate a scientifically defensible water quality guideline for a particular chemical (Emans et al 1993). The traditional approach to using single species toxicity data to protect aquatic ecosystems has been to apply arbitrary 'safety factors' to the lowest toxicity value for a particular chemical. The magnitude of these safety factors (usually in multiples of ten, ie 10, 100 etc) depends on whether acute or chronic toxicity data are available.

A safety factor of ten (Johnston 1991, Schudoma 1994), applied to the lowest toxicity value, is often used to take into account differences between lethal versus sublethal endpoints, short-term versus long-term exposures and median versus no-response concentrations. Safety factors also recognise that some untested species may be more sensitive, that there is uncertainty in extrapolating to the field, and also that test results are not a fixed number but a range of values with a degree of inherent uncertainty (Johnston 1991, Chapman 1992). Safety factors are routinely used in deriving water guideline values in many overseas countries (eg Canada) and formed the basis of the 1992 Australian water quality guidelines.

If a safety factor of ten is applied to the lowest U test result (ie BEC_{10}) for the collective U toxicity data on tropical freshwater biota (Appendix A) a guideline value of 5.6 μg UO_2 L^{-1} is calculated (ie green hydra; 56 μg L^{-1} divided by ten = 5.6 μg L^{-1}) or 4.9 μg U L^{-1}. This value is consistent with the interim guideline of 5 μg U L^{-1} currently in place for Magela Creek (NT DME 1982). If this procedure is repeated for Cu, a guideline value of 0.16 μg L^{-1} (ie hydra; 1.6 μg L^{-1} divided by ten = 0.16 μg L^{-1}) is calculated, well below the background Cu concentration (0.70 μg L^{-1}) measured in Magela Creek water. In this instance, the calculated value is not practical for use as a guideline. In situations such as this, the background total metal concentration should probably serve as the default guideline value. Common sense is clearly required when using safety factors to derive guideline values for the protection of aquatic life. This scenario highlights the importance of considering the speciation of a metal, rather than just total metal concentration (see Sections 5.2.4 & 6.1).

The use of safety factors remain a contentious issue amongst ecotoxicologists. Forbes and Forbes (1994) recently published a critique on the use of safety factors and distribution-based extrapolation models (Aldenberg & Slob 1993) in ecotoxicology. The most cited objection to the use of safety factors concerned their arbitrary and non-theoretical nature and the fact that they do not conform to risk assessment principles. Conversely, it has been argued that they are far less complex to use than the distribution-based extrapolation models. Although both approaches involve degrees of technical and value judgements, it has been argued that use of a risk-based approach allows informed debate about the level of protection that an aquatic ecosystem may require, and the statistical certainty with which that level of protection can be delivered. Sole reliance on the safety factor approach prevents any quantitative altering of protection levels and does not reflect the increased confidence in results with increasing certainty of toxicity data. The OECD (1992) recommended the use of safety factors primarily to derive interim water quality guidelines in the absence of adequate datasets for deriving guidelines using distribution-based extrapolation models. This recommendation will be adopted in the 1998 Australian water quality guidelines.

The derivation of water quality guidelines using distribution-based extrapolation models (ie risk-based approach), rather than relying solely on safety factors, is consistent with ecologically sustainable development principles (ie acceptance of some degree of environmental disturbance provided the integrity of the ecosystems is not threatened). This approach allows for some assessment of the degree of disturbance to an aquatic ecosystem

and whether a change in protection level for an interim water quality objective would give an acceptable level of ecosystem protection. Distribution-based extrapolation models have been used to derive water quality guidelines/criteria by Denmark (Samsoe-Peterson & Pederson 1995), the Netherlands (MHSPE 1994), South Africa (Roux et al 1996) and the USA (US EPA 1995a, 1995b). These are based on calculations of a statistical distribution of laboratory toxicity data and offer a pre-determined level of protection, usually 95% (Warne 1996). The use of risk-based and safety factor approaches for deriving water quality guidelines is critically reviewed by Warne (1997).

The derivation of the 1998 Australian (and New Zealand) water quality guidelines for toxicants relies primarily on the Dutch risk-based approach developed by Aldenberg and Slob (1993) and uses the ETX computer program (Aldenberg 1993). Toxicant guideline values are also calculated using the safety factor approach (modified from ANZECC (1992)) and the lowest value from the two methods is selected (ie following the OECD recommendation). This is consistent with the 'precautionary principle' (MacGarvin 1995), which is an integral part of preventative environmental protection.

The ETX program was employed to calculate risk-based guideline values for Cu and U using toxicity data for tropical freshwater biota given in Appendix A. All data were normalised to chronic NOEC values using extrapolation factors reported by Hendriks (1995) (eg a 96 h LC_{50} was divided by eight). The results are compared with those calculated using the safety factor approach in table 6. Both methods provide a similar guideline value for both metals, although the safety factor approach gives marginally lower values than the risk-based approach. If the precautionary principle is followed, guidelines for Cu and U should be based on the values derived using the safety factor approach. The practicality of these values have been discussed earlier. In summary, it is recommended that 1 μg Cu L^{-1} and 5 μg U L^{-1} be adopted as guideline values to protect Australian tropical freshwater biota.

Table 6 Derivation of water quality guidelines for Cu and U in Australian tropical freshwater systems

Metal	Risk-based approach[a]	Safety factor approach[b]
Cu (μg L^{-1})	0.35	0.16
U (μg L^{-1})	5.3 (6.0)[c]	4.9 (5.6)[c]

a Based on extrapolated chronic NOEC data with a 95% protection level with 95% certainty (Aldenberg 1993)

b Based on the lowest toxicity value reported divided by ten

c Concentrations expressed as uranyl (UO_2) (ie U x 1.14)

5.2.4 Speciation and bioavailability of U and Cu

Metal guidelines to protect aquatic biota are typically reported as a total metal concentration. However, it is well established that the bioavailability and toxicity of metals (and metalloids) to aquatic organisms are critically dependent on the physico-chemical form(s), or speciation, of these metals (Hamelink et al 1994, Tessier & Turner 1995). Metals may occur as a variety of physico-chemical forms in aquatic ecosystems; the free hydrated metal ion (M^{n+}) and metals complexed with a range of naturally occurring organic and inorganic compounds in soluble, colloidal or particulate forms (Pickering 1995). It is generally considered that metal toxicity is governed by the activity of the free hydrated metal ion or weak complexes that are able to dissociate at the cell membrane (ie the free-ion activity model, FIAM) (Campbell 1995). Metals in strong complexes or adsorbed to colloidal or particulate matter are usually considered less toxic (Hunt 1987). An exception to the FIAM are lipid-soluble metal complexes, which directly transverse the cell membrane to exert their toxicity (Tjälve &

Gottofrey 1991, Phinney & Bruland 1994). However, such complexes typically represent less than 1% of the total dissolved metal concentration. Overall, determination of metal speciation is essential in assessing the geochemical behaviour of metals in aquatic ecosystems, and hence, predicting their bioavailability and toxicity to biota.

To this end, the speciation of U and Cu in the test waters was predicted using the geochemical modelling code HARPHRQ. Figure 8 shows the percentage distribution of each U species plotted against total U concentration at pH 6. The free uranyl ion (UO_2^{2+}) constitutes a minor proportion of the total U concentration (ie 8% at 0.1 µg L^{-1} declining to 2% at 4000 µg L^{-1}). In contrast, polymeric uranyl species, such as $(UO_2)_2(OH)_3CO_3^-$, $(UO_2)_3(OH)_5^+$ and $(UO_2)_3(OH)_7^-$ increase in significance with increasing total U concentration (fig 8); this increase is compensated by a reduction in the relative proportions of monomeric uranyl species (ie $UO_2(OH)^+$ and UO_2CO_3).

Using regression analysis, Markich et al (1996) provided evidence that UO_2^{2+} and UO_2OH^+ are the dissolved U species primarily responsible (*ca.* 96%) for eliciting adverse behavioural responses in the freshwater bivalve *V. angasi*, between pH 5 and 6, where UO_2^{2+} is assigned twice the toxic effect of UO_2OH^+ (ie UO_2^{2+} is doubly charged, and hence, is able to bind two functional groups on membrane surfaces). These results provide the first evidence that the toxicity of U to biota is governed by the free uranyl ion (UO_2^{2+}), rather than the sum total of inorganic uranyl species or uranyl-organic species. This supports the FIAM (Campbell 1995). Therefore, in the context of this study (ie pH 6), the proportion of UO_2^{2+} (and UO_2OH^+) and, hence, the bioavailability of U to the test species, is considered low. However, Markich et al (1996) showed that the toxicity of U to *V. angasi* increased exponentially when pH was reduced from 6 to 5 (ie from an 48-h EC_{50} of 634 to 117 µg L^{-1}). Conversely, the authors demonstrated that the toxicity of U to *V. angasi* was substantially ameliorated with increasing concentration of DOC, in the form of a synthetic fulvic acid (ie from an 48-h EC_{50} of 634 to 1228 µg L^{-1}). Thus, there is a necessity to understand and quantify the influence of key water quality parameters, such as pH and DOC, on the toxicity of metals to freshwater biota.

The predicted speciation of Cu contrasts markedly to that of U. Unlike the complex speciation distribution predicted for U at pH 6, the speciation of Cu in the test water is relatively simple. Total Cu occurs predominantly (96%) as the free cupric ion (Cu^{2+}) with only a very small contribution (4%) from $CuOH^+$. The percentage contribution of both Cu species is predicted to remain constant for total Cu concentrations ranging from 1 to 100 µg L^{-1}. In contrast to U, Cu is predicted to be present in the test water in its most bioavailable, and hence, toxic form to freshwater biota. Further work will address the influence of water hardness, pH and DOC on the bioavailability, and hence, toxicity of Cu and U to *H. viridissima* and *M. mogurnda*. Additional information on the speciation and bioavailability of Cu and U in natural waters is reported elsewhere (Markich et al 1997).

Few chemical measurement techniques are currently available to determine the speciation of U in water. Physical separation, using ultrafiltration (with defined molecular weight cut-off), and analysis of the filtrate by ICPMS is probably the easiest method for determining U speciation, particularly to differentiate inorganic and organic uranyl complexes. However, this technique does not discriminate between inorganic uranyl species. Cathodic stripping voltammetry has been effectively used to study the speciation of U in estuarine and marine waters (van den Berg et al 1991), but has not yet been applied to freshwaters.

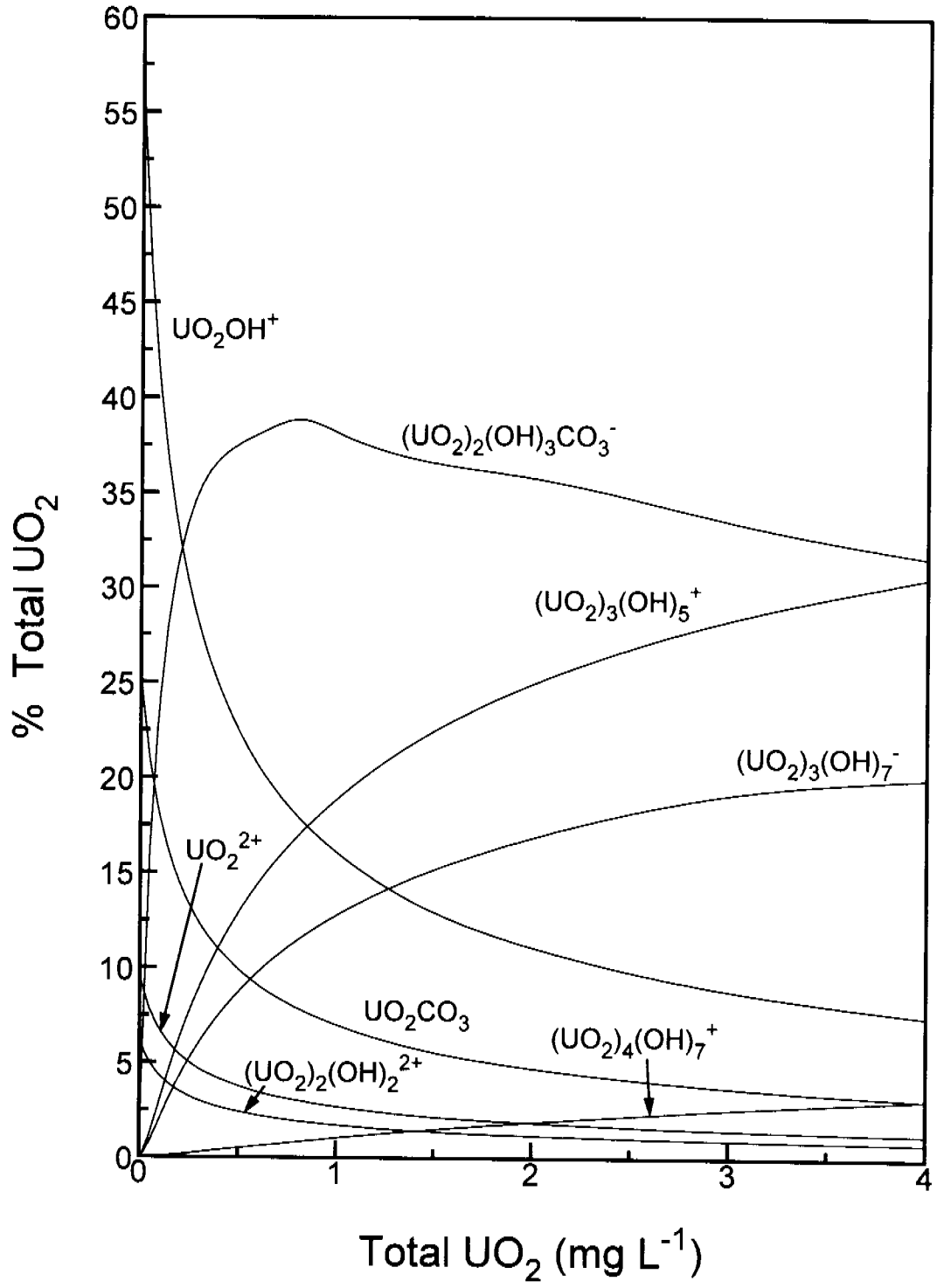

Figure 8 Predicted percentage speciation of U at pH 6 in the absence of organic ligands. Uranyl (UO_2) species comprising < 2% of total uranium were excluded for clarity.

This technique measures an operationally-defined labile fraction of U (ie free uranyl ion plus weakly complexed uranyl species). Time-resolved laser-induced fluorescence spectroscopy presently offers the best method for determining U speciation in water (Moulin 1995, Bernhard et al 1996). This technique has been shown to clearly discriminate between inorganic uranyl species (eg UO_2^{2+} and UO_2OH^+) across a range of pH values (pH 2–10). Another promising technique is UV-Vis spectroscopy (Meinrath et al 1996).

In contrast to U, a number of chemical measurement techniques are routinely available to determine the speciation of Cu in water. Of these, stripping voltammetry (anodic and cathodic) and potentiometry (Cu-ion selective electrode) are typically the most popular (Tessier & Turner 1995). A Cu-ion selective electrode was used in this study to measure the free cupric ion (Cu^{2+}) concentration at several Cu levels in the test waters. The results showed that the measured Cu^{2+} concentrations were very similar to those predicted using HARPHRQ (table 7), indicating that geochemical speciation modelling appears to be a valid approach for estimating Cu^{2+} in the test waters.

Table 7 Cupric ion (Cu^{2+}) concentrations in test water using two speciation techniques

Total copper concentration (µg L^{-1})	Cupric ion (Cu^{2+}) concentration (µg L^{-1})	
	Cu-ion selective electrode[a]	Geochemical modelling
5	5.02 ± 0.16	4.80
10	9.83 ± 0.12	9.60
20	19.4 ± 0.12	19.2
50	48.5 ± 0.31	48.0
100	97.2 ± 0.42	96.0

[a] mean ± standard error (n = 2)

6 General discussion

6.1 Incorporation of metal speciation and bioavailability into the Australian water quality guidelines

Toxicity data reported here for U and Cu were determined using a water quality that represents a high risk scenario (ie synthetic water with no organic chelators) in terms of the toxicity of each metal to the test organisms. However, maximum value will be obtained from these data when they are incorporated into a larger study, where key water quality variables, such as water hardness, pH and DOC, are considered. In freshwaters, water hardness has a significant effect on the bioavailability and, hence, toxicity of several metals (see review by Markich et al 1997). Although the 1992 Australian water quality guidelines (ANZECC 1992) acknowledge that the bioavailability of Cd Cu, Ni, Pb and Zn depends on water hardness, they provide no quantitative method for calculating metal guideline values, given a particular water hardness. The guidelines for Ni and Zn range from 15–150 mg L^{-1} and 5–50 mg L^{-1}, respectively, 'depending on hardness'. Such guidelines are of limited value to regulators and industry for determining the concentrations of both metals that will be of low risk to biota in a specific freshwater system (ie with a known hardness). This deficiency is addressed in the 1998 water quality guidelines by defining specific algorithms for a range of metals (Cd,

Cr(III), Cu, Ni, Pb and Zn) to permit calculation of hardness-modified guideline values (see Markich et al (1997) for further discussion).

A quantitative relationship between water hardness and a guideline value for the above mentioned metals already forms part of the national water quality guidelines in Canada (CCREM 1991, Porter et al 1995), South Africa (Roux et al 1996), the USA (USEPA 1995a, 1995b) and the UK (Gardiner & Zabel 1989). This relationship takes the form of a linear regression between the logarithm of toxicity endpoint values (eg 96 h LC_{50}) and the logarithm of water hardness (see US EPA 1978). As an example, the current Canadian water quality guidelines for Cd (Porter et al 1995) depend on water hardness as follows: at 30, 90, 150 and 210 mg L^{-1} (as $CaCO_3$), the guideline Cd concentrations are 0.01, 0.03, 0.05 and 0.06 $\mu g\ L^{-1}$, respectively.

Markich and Jeffree (1994) proposed that Ca concentration may be a better choice than total hardness (Ca + Mg) for the protection of freshwater biota, since Ca is far more effective at ameliorating metal toxicity than Mg. Indeed, in most freshwaters Ca is the prevalent (70%) hardness cation. The German water quality guidelines use Ca concentration instead of total hardness with Cu, Zn and Cd for the protection of freshwater fisheries (Rump & Krist 1992). Markich and Jeffree (1994) recommended that total hardness (Ca + Mg) will only be more useful when the concentration of Mg considerably exceeds that of Ca. As a pertinent example, the concentration of Mg typically exceeds that of Ca in fresh surface waters draining the Pine Creek Inlier (see Section 5.2.3) in tropical northern Australia (B Noller pers comm). Hence, both the Ca and Mg concentration are likely to be important in influencing the bioavailability and toxicity of metals in this region.

Apart from water (or Ca) hardness, pH and DOC (in the form of fulvic and humic acid) are important water quality parameters that may significantly effect the speciation and bioavailability of metals in freshwater systems (see review by Markich et al 1997). Similarly, salinity is considered to be the most relevant water quality parameter controlling metal bioavailability and toxicity in estuarine and marine waters (Hall & Anderson 1995). However, unlike water hardness, pH, DOC and salinity have not been used quantitatively to modify guideline values for metals. This stems primarily from (a) contradictory findings, especially in the case of pH, and/or (b) lack of good quality data to establish quantitative relationships (Markich et al 1997). The incorporation of pH, DOC and salinity, where applicable, into the Australian water quality guidelines, as modifiers of metal bioavailability and toxicity, will add significant value to the protection of freshwater biota on a site-specific basis, whilst allowing scope for the development of industry.

It is well recognised that water quality guidelines based on total metal concentration will be overprotective (US EPA 1995a), since only a fraction of the total concentration will be bioavailable, especially in samples containing appreciable concentrations of particulate matter. One approach recently promulgated by the US EPA (1995a) is the application of conversion factors to total metal concentrations to obtain dissolved metal concentrations. The US EPA (1995a) suggested that dissolved metal concentration more closely approximates the bioavailable concentration of a metal in the water column than does total metal concentration. However, most of the published conversion factors are very close to unity (eg Zn 0.978, Ni 0.998), indicating that the total and bioavailable metal concentrations are almost identical. For those metals where this applies (ie nine out of ten metals, Cr(III) being the exception) very little additional protection will be gained for aquatic biota by the use of a more complex evaluation process.

A different approach promulgated by the US EPA (1994) to amend national water quality criteria for site-specific conditions is the use of the 'water-effect ratio' (WER). The WER compares the toxicity of a chemical in the site water to its toxicity in laboratory water, for two or more aquatic species. Because metal toxicity in laboratory water is the basis of the national water quality criterion, the WER may be used as an adjustment to obtain a site-specific value. Adjustment may either increase or decrease the numeric value of the criterion. One shortcoming with this approach is the lack of consideration of any ecological census of receiving water conditions (eg rapid biological assessment procedures). This restricts the utility of the site-specific water quality criteria development. The rationale for conducting site-specific studies is to ensure that water quality guidelines for a body of water are neither over- nor under-protective for the local species, but rather that they adequately protect the structure and function of the aquatic community. The use of site-specific information to modify national water quality guidelines or criteria has an immediate appeal because it allows an investigator to change the focus from protecting a diverse range of ecosystems to a single receiving system.

Using a similar concept, metal speciation and bioavailability are incorporated into the 1998 Australian (and New Zealand) water quality guidelines using a risk-based, decision tree approach (Markich et al 1997), where a hierarchy of measurements of increasing complexity are prescribed that provide an increasingly detailed examination of specific metal species that are exerting the toxic effects. This approach will allow nominal guideline concentrations to be exceeded, provided that it can be demonstrated that the bioavailable fraction of the total metal concentration is below the guideline value, and hence, the risk of toxic effects is low.

A detailed discussion of the protocol used in the decision tree approach is given by Markich et al (1997). A summary is provided as follows. If the total (acid-soluble) metal concentration (ie water sample acidified to pH <2 at room temperature) exceeds the guideline value (modified for water hardness if the sample is a freshwater), then a measurement of the dissolved metal concentration should be made and compared with the same guideline value, on the assumption that the contribution of suspended particulate matter to metal bioavailability is low. While this assumption is generally true for some organisms (eg algae), other organisms, such as some species of molluscs and crustaceans, acquire metals from both the dissolved and particulate phases (see Markich at al 1997). Therefore, the bioavailability of particulate-bound metals also needs to be considered. This is incorporated into the sediment section of the 1998 Australian and New Zealand water quality guidelines.

The original (unacidified) water sample should be filtered, in the first instance, through a 0.45 μm membrane filter and then acidified (pH <2) at room temperature (ie dissolved (acid soluble) metal). Filtration through a 0.45 μm membrane is a traditional, yet practical, initial choice. Filtration through smaller pore-size membrane filters (down to 0.015 μm) is optional, but will invariably provide a better separation of the dissolved and particulate metal fraction. If the guideline value is still exceeded after filtration, then a more detailed consideration of speciation is required. This may include a range of techniques, including speciation modelling, chemical measurements and toxicity testing. The advantages and shortcomings of each of these techniques are discussed by Markich et al (1997).

At present, no universally applicable technique for determining metal bioavailability exists. Although toxicity testing provides a direct determination of metal bioavailability, it is labour intensive and very costly. Analytical measurements (eg ISE and ASV) that are specific for determining particular metal species (ie free metal ion and labile metal species) are also labour intensive and costly, but provide a more equivocal indication of metal bioavailability than direct toxicity testing. Geochemical speciation modelling using off-the-shelf packages

(eg MINTEQ) is perhaps the most cost-effective technique available, with an added advantage of providing a predictive capability, but suffers from being the most equivocal in determining metal bioavailability. Analytical measurements and speciation modelling may serve as valuable tools if performed in conjunction with toxicity tests, where particular metal species (eg free metal ion and labile metal species) can be related to a toxic response. These techniques are not trivial and should be only undertaken by appropriately experienced personnel.

The latest research has identified surface complexation of metals to cell membranes as a major determinant of metal bioavailability in aquatic organisms, and is attempting to predict metal bioavailability by incorporating the binding constants of metals with cell membranes (eg gills) into geochemical speciation models (Bergman & Dorward-King 1997). This approach aims to bridge the gap between speciation modelling and toxicity testing, but at present there are insufficient data for metals other than Cu and Cd.

In summary, the decision tree approach aims to assist regulators and industry make risk-based decisions on the appropriate level of environmental protection. There is a clear requirement to establish water quality guidelines that are easy to use by regulators and industry, yet which are scientifically defensible. Water quality guidelines should be used as important screening tools for agencies and individuals that have water assessment and management responsibilities. They provide one type of assessment tool that helps water managers to identify contaminants of concern, to prioritise areas of concern, and to implement appropriate environmental protection or enhancement strategies.

6.2 Suitability of the Australian water quality guidelines: Comparison of Australian and North American toxicity data

There is a clear requirement to assess the suitability of the Australian water quality guidelines with respect to Australian biota and environmental conditions. A major objective of this study was to collate all known metal toxicity data (Appendixes A and B) for aquatic biota in tropical Australia, as part of a broader study to test whether temperate North American metal toxicity data are applicable to the tropics of Australia. A comprehensive comparison of all metals and biota was beyond the scope of this study. However, a comparison of the toxicity of U and Cu to freshwater Crustacea and fish was performed (table 8). Uranium and Cu were selected because they provided the most comprehensive toxicity database for Australian tropical freshwater species. For comparative purposes, only toxicity data that were derived from studies using test waters with a total hardness and alkalinity of <30 mg L^{-1} (as $CaCO_3$) and a conductivity of <60 $\mu S\ cm^{-1}$ were considered. This process attempted to normalise for some of the major physico-chemical differences in the test waters between the two continents.

Few Cu toxicity data are available for tropical Australian crustacea (table 8). Only two species have been studied, the atyid shrimp *Caridina* sp. (pH 6) and the paleomonid shrimp *Macrobrachium* sp. (pH 7). In contrast, North American cructacea are dominated by water fleas, with only a small proportion of shrimps The mean 48 h LC_{50} values for Cu are very similar for both tropical Australian and temperate North American crustacea (table 8). The mean value for Australian crustacea falls within the 95% confidence interval of North American crustacea. The range of Cu toxicity values are very similar despite the concentrations of total hardness and alkalinity being approximately six times lower in the test waters used to derive Cu values for Australian crustacea Experimental studies have shown that *Caridina* sp. is about a factor of fifty times more sensitive to Cu than *Macrobrachium* sp. (Appendix A). However, only a small proportion (~10%) of this difference in sensitivity can be explained by Cu being less bioavailable at pH 7, as predicted using geochemical

speciation modelling. Clearly, further Cu toxicity data for tropical Australian species of crustacea are required before valid comparisons can be made.

Table 8 Comparative toxicity of Cu and U to freshwater Crustacea and Chordata (fish) from tropical Australia and temperate North America[a]

		Tropical Australia		Temperate North America	
Metal	Parameter	Crustacea[b]	Chordata[c] (Fish)	Crustacea[b]	Chordata[c] (Fish)
Cu	Mean	0.38 (24)[d]	1.6 (99)[d]	0.36 (23)[e]	0.96 (61)[e]
	95% CI	nc[f]	0.77–4.4 (49–277)	0.23–0.51 (14.7–32.4)	0.63–1.3 (40–81)
	Range	0.06–2.7 (3.5–170)	0.35–11.3 (22–718)	0.08–2.1 (5–133)	0.05–15.7 (3–1000)
	n	2	13	26	80
U	Mean	3.8 (995)[d]	9.4 (2530)[d]	13.4 (3610)[g]	15.3 (4120)[h]
	95% CI	nc[f]	6.6–12.1 (1780–3280)	nc[f]	4.1–34.0 (1100–9180)
	Range	1.7–5.4 (467–1470)	3.1–27.8 (830–7520)	nc[f]	6.2–32.9 (1670–8890)
	n	4	20	1	5

a Metal toxicity data (ie mean, 95% confidence interval and range) are expressed in μmol L^{-1} and μg L^{-1} (in parentheses). Geometric mean values were used for log-normal distributions. Presentation of the metal toxicity as a micromolar concentration normalises for differences in molecular mass between metals. Uranium (U) concentration is expressed as uranyl (ie UO_2). For comparative purposes, only toxicological data that were derived from studies using test waters with a total water hardness and alkalinity of <30 mg L^{-1} (as $CaCO_3$) and a conductivity of < 60 μS cm^{-1} were considered.

b A 24 h LC_{50} was used as the toxicological endpoint for U whereas a 48 h LC_{50} was used for Cu.

c A 96 h LC_{50} was used as the toxicological endpoint for U and Cu.

d Data were derived from references cited in Appendix A.

e Data were derived from references cited in US EPA water quality documents (US EPA 1978, 1985, 1995a).

f nc: not able to be calculated.

g Datum was derived from Kennedy et al (1995).

h Data were derived from Tarzwell & Henderson (1960), Davies (1980) and Parkhurst et al (1984).

Few U toxicity data are available for tropical Australian and North American crustacea (table 8). Only one North American study has been reported with soft water. The mean 24 h LC_{50} value for the North American water flea *Ceriodaphnia dubia* is a factor of 3.6 times higher than that reported for tropical Australian crustacea. The concentrations of total hardness and alkalinity were approximately six times lower, and the pH 1.1 log units lower (ie 6.6), in the test waters used for the Australian species of crustaceans. However, these differences in water chemistry are not sufficient to substantially increase the bioavailability of U, as predicted using geochemical speciation modelling (see Section 5.1.2).

An important water quality parameter not measured in studies from both continents was DOC. Dissolved organic carbon, in the form of fulvic and humic acid, is known to bind U strongly in solution, and hence, markedly ameliorate its toxicity to aquatic biota (Markich et al 1996). This parameter, singly, may be capable of explaining the measured differences in the mean toxicity of U to the test organisms, if sufficient differences in DOC concentration existed between the two sets of studies. Although the toxicity of U to water fleas was compared for both continents, only one North American species was studied. Thus, no data are available to indicate the range of sensitivities to U for North American crustacea. It is clear that additional U toxicity data are required from both continents, particular North America, before valid comparisons can be made.

The Cu toxicity data for fish in both continents covers a broad range of species, and possibly offers the best dataset for comparison. Eleven species of fish from tropical Australia have been studied (Appendix A), whereas seventeen species of fish have been studied from temperate North America (US EPA 1978, 1985, 1995a). The mean 96 h LC_{50} value of Cu for North American fish is a factor of 1.6 times lower than that for tropical Australian fish (table 8). However, fish from both continents shown a large degree of variability in their sensitivity to Cu (eg 330-fold difference for North American species). Hence, considerable overlap in the range of 96 h LC_{50} values is evident.

The range of 96 h LC_{50} values for U are similar for both tropical Australian and temperate North American fish (table 8). Although the mean 96 h LC_{50} value of U for tropical Australian fish is a factor of 1.6 times lower than that for North American fish, it falls within the large 95% confidence interval of the latter. The five toxicity values available for North American fish derive from only three species, the fathead minnow (*Pimephales promelas*), brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*). These three species of fish show a large degree of variability in their sensitivity to U under similar physico-chemical conditions. In contrast, the toxicity of U to ten species of tropical Australian fish has been studied. An order of magnitude difference in the relative sensitivity of U to these fish has been shown. Additional U toxicity data are required for temperate North American species of fish before valid comparisons can be made.

In general, data on U toxicity are few, particularly for North American species. Additional Cu toxicity data for tropical Australian species of crustaceans are required before reliable comparisons can be made with North American species. A clear conclusion from the above comparisons is that freshwater species of crustacea and fish from tropical Australia have a similar range of sensitivity to U and Cu as those species from temperate North America. These findings are consistent with the results of several other Australian studies that have assessed the relative sensitivity of Australian and northern hemisphere freshwater biota to various chemicals. Skidmore and Firth (1983) showed that there was considerable overlap in the range of sensitivities of Australian and North American fish and Crustacea to Cu and Zn. Johnston et al (1990) investigated the acute and chronic toxicity of phenol, pentachlorophenol and 1,1,2 trichloroethane to both Australian and northern hemisphere species of fish and Crustacea. They concluded that the range of sensitivities for species within each phyla were similar once important differences in water chemistry, such as temperature and salinity, were considered.

Davies et al (1994) studied the acute sublethal responses of several species of fish and Crustacea to seven pesticides (acephate, fenitrothion, cypermethrin, MPCA-Na, atrazine, cyanazine and clorothalonil). Their results showed that the toxicity of pesticides to both animal phyla were within the range reported for northern hemisphere species. The acute toxicity of endosulfan has been studied for both Australian and northern hemisphere species of fish (Sunderam et al 1992, R Patra pers comm) and Crustacea (Sunderam et al 1994, Patra et al 1996). These studies confirmed that the range of sensitivity of Australian species to endosulfan was similar to those from the northern hemisphere at moderate temperatures (up to about 20°C). However, the sensitivity of Australian Crustacea to endosulfan increased exponentially between 20 and 30°C (Patra et al 1996, R Patra pers comm), resulting in toxicity values lower than those reported for northern hemisphere species, but comparable with Southern African species (Magdza 1983).

In summary, it can be concluded that the Australian water quality guidelines, derived largely from North America toxicological data, are appropriate for Australian species and conditions when key water quality variables (eg temperature, water hardness and alkalinity) are

considered. This assertion can be tested further as more Australian toxicity data are generated and/or compiled. It is therefore proposed that North American toxicity data be used to derive Australian water quality guidelines for protecting aquatic life in situations where toxicity data are either absent or scant for Australian aquatic biota. North American toxicity data should be replaced by Australian toxicity data when available.

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APPENDIXES

Appendix A Summary of metal toxicity data for Australian tropical freshwater biota[a]

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity ($\mu S\ cm^{-1}$)	Hardness (mg $CaCO_3\ L^{-1}$)	Alkalinity (mg $CaCO_3\ L^{-1}$)	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration ($\mu g\ L^{-1}$)[f]	Water concentration ($\mu mol\ L^{-1}$)	Concentration (measured or nominal)	Reference
Cnidaria																
Green hydra (*Hydra viridissima*)	Copper	Adult	Static-renewal	Synthetic Magela Creek water	27 ± 1	6.0 ± 0.1	23 (22–24)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Population growth	96	1.6 (BEC_{10})[c] 1.8 (MDEC)[d] 4.0 (EC_{50})[e] (3.8–4.2)	0.03 (BEC_{10})[c] 0.03 (MDEC)[d] 0.06 (EC_{50})[e] (0.06–0.07)	Measured	Markich & Camilleri (This study)
	Uranium	Adult	Static-renewal	Buffalo Billabong water	30 ± 1	6.5 ± 0.2	26 (24–28)	4[f] (3–5)	3[f] (2–4)	NR[g]	Population growth	96	160 (LOEC)[h] (Dry season) 194 (LOEC) (Wet season)	0.59 (LOEC)[h] (Dry season) 0.72 (LOEC) (Wet season)	Measured	***eriss*** (Unpubl)
	Uranium	Adult	Static-renewal	Synthetic Magela Creek water	27 ± 1	6.0 ± 0.1	23 (22–24)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Population growth	96	56 (BEC_{10}) 61 (MDEC) 108 (EC_{50}) (102–114)	0.21 (BEC_{10}) 0.23 (MDEC) 0.40 (EC_{50}) (0.38–0.42)	Measured	Markich & Camilleri (This study)
Pink hydra (*Hydra vulgaris*)	Uranium	Adult	Static-renewal	Buffalo Billabong water	30 ± 1	6.4 ± 0.1	22 (23–24)	4[f] (3–5)	3[f] (2–4)	NR	Population growth	96	740 (LOEC) (Dry season)	2.7 (LOEC) (Dry season)	Measured	***eriss*** (Unpubl)
Mollusca																
Mussel (*Velesunio angasi*)	Copper	Adult	Static-renewal	Tap water	25 ± 1	7.5 ± 0.1	150[d] (145–155)	54[f] (51–57)	27[f] (25–29)	NR	Survival	96	21000 (LC_{50})[j] (13000–32000)	330 (LC_{50})[j] (205–504)	Nominal	Skidmore & Firth (1983)
												792 (33 d)	420 (LC_{50})	6.61 (LC_{50})		Skidmore (1986)
												1320 (55 d)	~ 210 (LC_{50})	~ 3.31 (LC_{50})		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	9220 (BEC_{10})[k] 17870 (EC_{50}) (14250–21490)	168(BEC_{10})[k] 325 (EC_{50}) (259–391)	Measured	Markich (Unpubl)
													17200 (BEC_{10})[l] 30000 (EC_{50}) (28500–31500)	313 (BEC_{10})[l] 546 (EC_{50}) (519–573)		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	3.7 (D)	Behaviour	48	10310 (BEC_{10})[k] 18500 (EC_{50}) (15400–21600)	188 (BEC_{10})[k] 337 (EC_{50}) (280–393)	Measured	Markich (Unpubl)
													16500 (BEC_{10})[l] 28300 (EC_{50}) (26900–29700)	300 (BEC_{10})[l] 515 (EC_{50}) (490–541)		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	8.9 (D)	Behaviour	48	10000 (BEC_{10})[k] 18720 (EC_{50}) (15370–22070)	182 (BEC_{10})[k] 341 (EC_{50}) (280–402)	Measured	Markich (Unpubl)
													17100 (BEC_{10})[l] 29400 (EC_{50}) (28000–30800)	311 (BEC_{10})[l] 535 (EC_{50}) (510–561)		

Appendix A Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[j]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Mussel (*Velesunio angasi*)	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.3 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	9190 (BEC_{10})[k] 18530 (EC_{50}) (14560–22500)	167 (BEC_{10})[k] 337 (EC_{50}) (265–410)	Measured	Markich (Unpubl)
													16800 (BEC_{10})[l] 29500 (EC_{50}) (28000–31000)	306 (BEC_{10})[l] 537 (EC_{50}) (510–564)		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.5 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	10010 (BEC_{10})[k] 19980 (EC_{50}) (15050–22910)	182 (BEC_{10})[k] 364 (EC_{50}) (274–417)	Measured	Markich (Unpubl)
													16500 (BEC_{10})[l] 28700 (EC_{50}) (27200–30200)	300 (BEC_{10})[l] 522 (EC_{50}) (495–550)		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.5 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	3.7 (D)	Behaviour	48	9360 (BEC_{10})[k] 18310 (EC_{50}) (14790–21830)	170 (BEC_{10})[k] 333 (EC_{50}) (269–397)	Measured	Markich (Unpubl)
													15800 (BEC_{10})[l] 27200 (EC_{50}) (25700–28700)	288 (BEC_{10})[l] 495 (EC_{50}) (468–522)		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.5 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	8.9 (D)	Behaviour	48	10200 (BEC_{10})[k] 19520 (EC_{50}) (15860–23180)	186 (BEC_{10})[k] 355 (EC_{50}) (289–422)	Measured	Markich (Unpubl)
													16800 (BEC_{10})[l] 28800 (EC_{50}) (27400–30200)	306 (BEC_{10})[l] 524 (EC_{50}) (499–550)		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.8 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	10040 (BEC_{10})[k] 18490 (EC_{50}) (15030–21950)	183 (BEC_{10})[k] 337 (EC_{50}) (274–399)	Measured	Markich (Unpubl)
													16300 (BEC_{10})[l] 27900 (EC_{50}) (26500–29300)	297 (BEC_{10})[l] 508 (EC_{50}) (482–533)		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	6.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	10000 (BEC_{10})[k] 19100 (EC_{50}) (15300–22900)	182 (BEC_{10})[k] 348 (EC_{50}) (278–417)	Measured	Markich (Unpubl)
													16000 (BEC_{10})[l] 27500 (EC_{50}) (26100–28900)	291 (BEC_{10})[l] 501 (EC_{50}) (475–526)		

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Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[j]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Mussel (*Velesunio angasi*)	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	6.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	3.7 (D)	Behaviour	48	10100 (BEC_{10})[k] 19180 (EC_{50}) (15460–22900)	184 (BEC_{10})[k] 349 (EC_{50}) (281–417)	Measured	Markich (Unpubl)
													16100 (BEC_{10})[l] 27700 (EC_{50}) (26300–29100)	293 (BEC_{10})[l] 504 (EC_{50}) (479–530)		
	Manganese	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	6.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	8.9 (D)	Behaviour	48	10040 (BEC_{10})[k] 19340 (EC_{50}) (15750–22990)	183 (BEC_{10})[k] 352 (EC_{50}) (287–418)	Measured	Markich (Unpubl)
													16300 (BEC_{10})[l] 27900 (EC_{50}) (26500–29300)	297 (BEC_{10})[l] 508 (EC_{50}) (482–533)		
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	62 (BEC_{10})[k] 89 (EC_{50}) (83–95)	0.23 (BEC_{10})[k] 0.33 (EC_{50}) (0.31–0.35)	Measured	Markich (Unpubl)
													92 (BEC_{10})[l] 117 (EC_{50}) (113–121)	0.34 (BEC_{10})[l] 0.43 (EC_{50}) (0.42–0.45)		Markich et al (1996)
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	3.7 (D)	Behaviour	48	78 (BEC_{10})[k] 112 (EC_{50}) (101–123)	0.29 (BEC_{10})[k] 0.41 (EC_{50}) (0.37–0.46)	Measured	Markich (Unpubl)
													113 (BEC_{10})[l] 144 (EC_{50}) (138–150)	0.42 (BEC_{10})[l] 0.53 (EC_{50}) (0.51–0.56)		Markich et al (1996)
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	8.9 (D)	Behaviour	48	138 (BEC_{10})[k] 194 (EC_{50}) (188–200)	0.51 (BEC_{10})[k] 0.72 (EC_{50}) (0.70–0.74)	Measured	Markich (Unpubl)
													197 (BEC_{10})[l] 247 (EC_{50}) (240–254)	0.73 (BEC_{10})[l] 0.91 (EC_{50}) (0.89–0.94)		Markich et al (1996)
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.3 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	73 (BEC_{10})[k] 106 (EC_{50}) (96–116)	0.27 (BEC_{10})[k] 0.39 (EC_{50}) (0.36–0.43)	Measured	Markich (Unpubl)
													108 (BEC_{10})[l] 141 (EC_{50}) (135–147)	0.40 (BEC_{10})[l] 0.52 (EC_{50}) (0.50–0.54)		Markich et al (1996)

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Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[i]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Mussel (*Velesunio angasi*)	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.5 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	85 (BEC_{10})[k] 126 (EC_{50}) (114–138)	0.31 (BEC_{10})[k] 0.47 (EC_{50}) (0.42–0.51)	Measured	Markich (Unpubl)
													125 (BEC_{10})[l] 163 (EC_{50}) (156–170)	0.46 (BEC_{10})[l] 0.60 (EC_{50}) (0.58–0.63)		Markich et al (1996)
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.5 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	3.7 (D)	Behaviour	48	133 (BEC_{10})[k] 190 (EC_{50}) (184–196)	0.49 (BEC_{10})[k] 0.70 (EC_{50}) (0.68–0.73)	Measured	Markich (Unpubl)
													192 (BEC_{10})[l] 242 (EC_{50}) (233–251)	0.71 (BEC_{10})[l] 0.90 (EC_{50}) (0.86–0.93)		Markich et al (1996)
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.5 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	8.9 (D)	Behaviour	48	291 (BEC_{10})[k] 399 (EC_{50}) (381–417)	1.1 (BEC_{10})[k] 1.5 (EC_{50}) (1.4–1.5)	Measured	Markich (Unpubl)
													399 (BEC_{10})[l] 497 (EC_{50}) (477–517)	1.5 (BEC_{10})[l] 1.8 (EC_{50}) (1.8–1.9)		Markich et al (1996)
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	5.8 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	130 (BEC_{10})[k] 210 (EC_{50}) (191–229)	0.48 (BEC_{10})[k] 0.78 (EC_{50}) (0.70–0.85)	Measured	Markich (Unpubl)
													214 (BEC_{10})[l] 290 (EC_{50}) (275–303)	0.79 (BEC_{10})[l] 1.1 (EC_{50}) (1.0–1.1)		Markich et al (1996)
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	6.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Behaviour	48	244 (BEC_{10})[k] 446 (EC_{50}) (427–465)	0.90 (BEC_{10})[k] 1.7 (EC_{50}) (1.6–1.7)	Measured	Markich (Unpubl)
													416 (BEC_{10})[l] 634 (EC_{50}) (606–662)	1.5 (BEC_{10})[l] 2.3 (EC_{50}) (2.2–2.5)		Markich et al (1996)
	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	6.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	3.7 (D)	Behaviour	48	362 (BEC_{10})[k] 597 (EC_{50}) (559–635)	1.3 (BEC_{10})[k] 2.2 (EC_{50}) (2.1–2.4)	Measured	Markich (Unpubl)
													558 (BEC_{10})[l] 824 (EC_{50}) (786–862)	2.1 (BEC_{10}) 3.1 (EC_{50}) (2.9–3.2)		Markich et al (1996)

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Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[j]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Mussel (*Velesunio angasi*)	Uranium	Adult	Flow-through	Synthetic Magela Creek water	28.0 ± 0.1	6.0 ± 0.1	26 (25–27)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	8.9 (D)	Behaviour	48	635 (BEC_{10})[k] 941 (EC_{50}) (888–994)	2.4 (BEC_{10})[k] 3.5 (EC_{50}) (3.3–3.7)	Measured	Markich (Unpubl)
													913 (BEC_{10})[l] 1228 (EC_{50}) (1188–1268)	3.4 (BEC_{10})[l] 4.5 (EC_{50}) (4.4–4.7)		Markich et al (1996)
Crustacea																
Shrimp (*Caridina* sp.)	Copper	Adult	Flow-through	Synthetic Gulungul Creek water	27.0 ± 0.5	6.0 ± 0.1	NR	27 (25–30)	5	< 0.5 (T)	Survival	48	4.5 (LC_{50}) (2–8)	0.071 (LC_{50}) (0.031–0.126)	Measured	Williams et al (1991)
												72	4 (LC_{50}) (2–6)	0.063 (LC_{50}) (0.031–0.094)		
												96	3.5 (LC_{50}) (2–5)	0.055 (LC_{50}) (0.031–0.079)		
Water flea (*Dadaya macrops*)	Uranium	Neonate (< 6 h)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	24	1254 (LC_{50}) (923–1664)	4.64 (LC_{50}) (3.42–6.16)	Measured	Bywater et al (1991)
Water flea (*Diaphanosoma excisum*	Uranium	Neonate (< 6 h)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	24	1140 (LC_{50}) (787–1573)	4.22 (LC_{50}) (2.91–5.83)	Measured	Bywater et al (1991)
Water flea (*Latonopsis fasciculata*)	Uranium	Neonate (< 6 h)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	24	467 (LC_{50}) (365–593)	1.73 (LC_{50}) (1.35–2.20)	Measured	Bywater et al (1991)
Prawn (*Macrobrachium* sp.)	Copper	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	170 (LC_{50})	2.68 (LC_{50})	Nominal	Giles (1974)
	Copper	Adult	Static-renewal	Tap water	25 ± 1	7.5 ± 0.1	150 (145–155)	54 (51–57)	27 (25–29)	NR	Survival	48	170 (LC_{50})	2.68 (LC_{50})	Nominal	Skidmore & Firth (1983)
												96	160 (LC_{50})	2.52 (LC_{50})		
	Lead	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	500 (LC_{50})	2.41 (LC_{50})	Nominal	Giles (1974)
	Uranium	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	> 5700 (LC_{50})	> 21.11 (LC_{50})	Nominal	Giles (1974)
	Zinc	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	430 (LC_{50})	6.58 (LC_{50})	Nominal	Giles (1974)
Water flea (*Moinodaphnia macleayi*)	Uranium	Neonate (< 6 h)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	24	1470 (LC_{50}) (1210–1770)	5.44 (LC_{50}) (4.48–6.55)	Measured	Bywater et al (1991)

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Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[i]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Water flea (*Moinodaphnia macleayi*)	Uranium	Neonate (< 6 h)	Static-renewal	Magela Creek water	27 ± 1	6.5 ± 0.1	22	4[f] (3–5)	3[f] (2–4)	NR	Survival	48	211 (LC_{50}) (200–222)	0.78 (LC_{50}) (0.74–0.82)	Measured	***eriss*** (Unpubl)
	Uranium	Neonate (< 6 h)	Static-renewal	Magela Creek water	27 ± 1	6.5 ± 0.1	22	4[f] (3–5)	3[f] (2–4)	NR	Reproduction	120	20 (NOEC)[m] 22 (LOEC) 44 (EC_{50}) (41–47)	0.074 (NOEC)[m] 0.081 (LOEC) 0.163 (EC_{50}) (0.15–0.17)	Measured	***eriss*** (Unpubl)
Chordata																
Chanda perch (*Ambassis castelnaui*)	Copper	Adult	Static-renewal	Tap water	25 ± 1	7.5 ± 0.1	150 (145–155)	54 (51–57)	27 (25–29)	NR	Survival	48	200 (LC_{50})	3.2 (LC_{50})	Nominal	Skidmore (1986)
												96	140 (LC_{50})	2.2 (LC_{50})		
												192	120 (LC_{50})	1.9 (LC_{50})		
	Zinc	Adult	Static-renewal	Tap water	25 ± 1	7.5 ± 0.1	150 (145–155)	54 (51–57)	27 (25–29)	NR	Survival	48	4300 (LC_{50})	65.8 (LC_{50})	Nominal	Skidmore & Firth (1983)
												96	3900 (LC_{50})	59.7 (LC_{50})		
												192	3900 (LC_{50})	59.7 (LC_{50})		
Reticulated perch (*Ambassis macleayi*)	Uranium	Juvenile	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	910 (LC_{50}) (627–1230)	3.4 (LC_{50}) (2.3–4.6)	Measured	Bywater et al (1991)
												72	910 (LC_{50}) (627–123)	3.4 (LC_{50}) (2.3–4.6)		
												96	910 (LC_{50}) (627–123)	3.4 (LC_{50}) (2.3–4.6)		
Striped grunter (*Amniataba percoides*)	Uranium	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	2850 (LC_{50})	10.6 (LC_{50})	Nominal	Giles (1974)
	Zinc	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	200 (LC_{50})	3.1 (LC_{50})	Nominal	Giles (1974)
Mariana's hardyhead (*Craterocephalus marianae*)	Uranium	Juvenile	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	2120 (LC_{50}) (1642–2497)	7.9 (LC_{50}) (6.1–9.3)	Measured	Bywater et al (1991)
												72	1390 (LC_{50}) (935–1835)	5.2 (LC_{50}) (3.5–6.8)		
												96	1390 (LC_{50}) (935–1835)	5.15 (LC_{50}) (3.5–6.8)		
Marjorie's hardyhead (*Craterocephalus marjoriae*)	Copper	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	40 (LC_{50})	0.63 (LC_{50})	Nominal	Giles (1974)

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Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[i]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Marjorie's hardyhead (*Craterocephalus marjoriae*)	Lead	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	180 (LC_{50})	0.87 (LC_{50})	Nominal	Giles (1974)
	Manganese	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	10200 (LC_{50})	186 (LC_{50})	Nominal	Giles (1974)
	Uranium	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	4845 (LC_{50})	17.9 (LC_{50})	Nominal	Giles (1974)
	Zinc	Adult	Static	Magela Creek water	25 ± 1	7.0 ± 0.1	NR	10	NR	NR	Survival	96	140 (LC_{50})	2.14 (LC_{50})	Nominal	Giles (1974)
Fly-specked hardyhead (*Craterocephalus stercusmuscarum*)	Copper	Adult	Static	Buffalo Billabong water	27 ± 1	6.9 ± 0.1	NR	6.6	NR	NR	Survival	96	17 (LC_{50}) (16–27)	0.27 (LC_{50}) (0.25–0.42)	Nominal	Baker & Walden (1984)
	Zinc	Adult	Static	Buffalo Billabong water	27 ± 1	7.2 ± 0.1	42 (38–46)	42	NR	NR	Survival	96	600 (LC_{50}) (340–1070)	9.2 (LC_{50}) (5.2–16.4)	Nominal	Baker & Walden (1984)
Penny fish (*Denariusa bandata*)	Copper	Adult	Flow-through	Synthetic Gulungul Creek water	27.0 ± 0.5	6.0 ± 0.1	NR	27 (25–30)	5	< 0.5 (T)	Survival	48	140 (LC_{50}) (105–195)	2.20 (LC_{50}) (1.65–3.07)	Measured	Williams et al (1991)
												72	120 (LC_{50}) (88–170)	1.89 (LC_{50}) (1.38–2.68)		
												96	77 (LC_{50}) (42–120)	1.21 (LC_{50}) (0.66–1.89)		
Carp gudgeon (*Hypseleotris compressus*)	Cadmium	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	10000 (LC_{50})	89.0 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Copper	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	330 (LC_{50})	5.2 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Lead	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	13000 (LC_{50})	62.7 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Manganese	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	> 500000 (LC_{50})	> 9100 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Uranium	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	7520 (LC_{50})	27.9 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)

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Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[i]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Carp gudgeon (*Hypseleotris compressus*)	Zinc	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	9700 (LC_{50})	148 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
Spangled grunter (*Madigania unicolor*)	Uranium	Adult	Static	Magela Creek water	25 ± 2	7.0 ± 0.1	NR	10	NR	NR	Survival	96	4670 (LC_{50})	17.3 (LC_{50})	Nominal	Giles (1974)
Black-striped rainbowfish (*Melanotaenia nigrans*)	Cadmium	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	1500 (LC_{50})	13.4 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Copper	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	230 (LC_{50})	3.6 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Copper	Adult	Static	Buffalo Billabong water	27 ± 1	7.2 ± 0.2	35	25	18	NR	Survival	96	120 (LC_{50}) (100–140)	1.9 (LC_{50}) (1.6–2.2)	Nominal	Baker & Walden (1984)
	Lead	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	10000 (LC_{50})	48.3 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Manganese	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	> 500000 (LC_{50})	> 9100 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Uranium	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	5130 (LC_{50})	19.0 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Uranium	Larvae (7 d)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	2400 (LC_{50}) (1900–2780)	8.9 (LC_{50}) (7.1–10.3)	Measured	Bywater et al (1991)
												72	2140 (LC_{50}) (1425–2930)	7.9 (LC_{50}) (5.3–10.9)		
												96	1940 (LC_{50}) (1410–1585)	7.2 (LC_{50}) (5.2–5.90)		
	Uranium	Adult (90 d)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	2700 (LC_{50}) (1870–3510)	10.0 (LC_{50}) (6.9–13.0)	Measured	Bywater et al (1991)
												72	2250 (LC_{50}) (1810–2670)	8.3 (LC_{50}) (6.7–9.9)		
												96	2160 (LC_{50}) (1740–2600)	8.0 (LC_{50}) (6.5–9.6)		
	Zinc	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	> 10000 (LC_{50})	> 153 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)

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Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[j]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Black-striped rainbowfish (*Melanotaenia nigrans*)	Zinc	Adult	Static	Magela Creek water	27 ± 1	6.8 ± 0.5	40 (36–40)	22	15	NR	Survival	96	13900 (LC_{50}) (10000–15900)	213 (LC_{50}) (153–243)	Nominal	Baker & Walden (1984)
Chequered rainbowfish (*Melanotaenia splendida inornata*)	Cadmium	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	18000 (LC_{50})	160 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Copper	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	720 (LC_{50})	11.3 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Copper	Adult	Static	Magela Creek water	27 ± 1	6.9 ± 0.1	29	3.3	6.7	NR	Survival	96	60 (LC_{50}) (40–90)	0.94 (LC_{50}) (0.63–1.4)	Nominal	Baker & Walden (1984)
	Copper	Adult	Static-renewal	Tap water	25 ± 1	7.5 ± 0.1	150 (145–155)	54 (51–57)	27 (25–29)	NR	Survival	96	460 (LC_{50})	7.2 (LC_{50})	Nominal	Skidmore (1986)
												192	340 (LC_{50})	5.4 (LC_{50})		
											Reproduction	720 (30 d)	18 (LOEC)	0.28 (LOEC)		
													12 (NOEC)	0.19 (NOEC)		
	Copper	Adult	Flow-through	Synthetic Gulungul Creek water	27.0 ± 0.5	6.0 ± 0.1	NR	27 (25–30)	5	< 0.5 (T)	Survival	48	210 (LC_{50}) (175–250)	3.3 (LC_{50}) (2.8–3.9)	Measured	Williams et al (1991)
												72	205 (LC_{50}) (175–240)	3.2 (LC_{50}) (2.8–3.8)		
												96	168 (LC_{50}) (140–195)	2.6 (LC_{50}) (2.2–3.1)		
	Lead	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	32000 (LC_{50})	154 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Manganese	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	> 500000 (LC_{50})	> 9100 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Uranium	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	6840 (LC_{50})	25.3 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Uranium	Larvae (7 d)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	3140 (LC_{50}) (2590–3830)	11.6 (LC_{50}) (9.6–14.2)	Measured	Bywater et al (1991)
												72	3030 (LC_{50}) (2470–3740)	11.2 (LC_{50}) (9.2–13.9)		
												96	3030 (LC_{50}) (2470–3740)	11.2 (LC_{50}) (9.2–13.9)		

Appendix A Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[j]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Chequered rainbowfish (*Melanotaenia splendida inornata*)	Uranium	Adult (90 d)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	4380 (LC_{50}) (2975–8730)	16.2 (LC_{50}) (11.0–32.3)	Measured	Bywater et al (1991)
												72	3940 (LC_{50}) (2680–7490)	14.6 (LC_{50}) (9.9–27.7)		
												96	3944 (LC_{50}) (2680–7490)	14.6 (LC_{50}) (9.9–27.7)		
	Uranium	Larvae (14 d)	Flow-through	Buffalo Billabong water	30 ± 0.1	6.6 ± 0.2	38 (36–40)	5.1	3.2	5.8 (D)	Survival	96	1585 (LC_{50}) (1250–2000)	5.9 (LC_{50}) (4.64–7.39)	Measured	Holdway (1992)
	Uranium	Juvenile (31 d)	Flow-through	Buffalo Billabong water	30 ± 0.1	6.3 ± 0.2	36 (34–38)	4.1 (4.0–4.2)	1.8 (1.7–1.9)	1.5 (< 0.1–4)[d] (D) 2.7 (0.8–4.6)[d] (T)	Survival	168	1790 (LC_{50}) (1540–2420)	6.6 (LC_{50}) (5.7–8.9)	Measured	Holdway (1992)
	Zinc	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	38000 (LC_{50})	581 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Zinc	Adult	Static	Magela Creek water	27 ± 1	7.0 ± 0.2	33 (32–34)	27	NR	NR	Survival	96	6200 (LC_{50}) (5200–7400)	95 (LC_{50}) (80–113)	Nominal	Baker & Walden (1984)
Chequered rainbowfish (*Melanotaenia splendida splendida*)	Copper	Adult	Static-renewal	Tap water	25 ± 1	7.5 ± 0.1	150 (145–155)	54 (51–57)	27 (25–29)	NR	Survival	96	200 (LC_{50})	3.15 (LC_{50})	Nominal	Skidmore & Firth (1984)
Purple-spotted gudgeon (*Mogurnda mogurnda*)	Copper	Embryo/ Sac-fry	Static-renewal	Buffalo Billabong water	30 ± 1[f]	6.5[f]	NR	4[f] (3–5)	3[f] (2–4)	NR	Survival (Sac-fry)	96	20 (NOEC) 64 (LOEC)	0.31(NOEC) 1.0 (LOEC)	Nominal	Rippon & Hyne (1992)
											Hatching (Embryo)	120	> 200 (LOEC)	> 3.2 (LOEC)		
	Copper	Sac-fry (1 d)	Static-renewal	Synthetic Magela Creek water	27 ± 1	6.0 ± 0.1	23 (22–24)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Survival	96	12 (BEC_{10}) 13 (MDEC) 23 (LC_{50}) (22–24)	0.19 (BEC_{10}) 0.20 (MDEC) 0.36 (LC_{50}) (0.35–0.38)	Measured	Markich & Camilleri (This study)
	Uranium	Larvae (7 d)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	2340 (LC_{50}) (1860–2790)	8.7 (LC_{50}) (6.9–10.3)	Measured	Bywater et al (1991)
												72	1265 (LC_{50}) (950–1650)	4.7 (LC_{50}) (3.5–6.1)		
												96	1265 (LC_{50}) (950–1650)	4.7 (LC_{50}) (3.5–6.1)		

Appendix A Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[j]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Purple-spotted gudgeon (*Mogurnda mogurnda*)	Uranium	Adult (90 d)	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	2450 (LC_{50}) (1960–2990)	9.1 (LC_{50}) (7.3–11.1)	Measured	Bywater et al (1991)
												72	1665 (LC_{50}) (1280–2170)	6.2 (LC_{50}) (4.7–8.0)		
												96	1665 (LC_{50}) (1280–2170)	6.2 (LC_{50}) (4.7–8.0)		
	Uranium	Larvae (1 d)	Flow-through	Buffalo Billabong water	27 ± 0.1	6.4 ± 0.1	29 (28–30)	3.2 (3.0–3.4)	3 (2.8–3.2)	5.1 (4.5–5.7) (D) 5.4 (4.8–6.0) (T)	Survival	336	1000 (NOEC) 2040 (LOEC)	3.7 (NOEC) 7.6 (LOEC)	Measured	Holdway (1992)
												336 (+ 360 h post exposure)	502 (NOEC) 1000 (LOEC)	1.6 (NOEC) 3.7 (LOEC)		
	Uranium	Larvae (1 d)	Flow-through	Buffalo Billabong water	30 ± 0.1	6.3 ± 0.2	36 (34–38)	4.1 (4.0–4.2)	1.8 (1.7–1.9)	1.5 (< 0.1–4) (D) 2.7 (0.8–4.6) (T)	Survival	168	1810 (LC_{50}) (1730–1780)	6.7 (LC_{50}) (6.4–6.6)	Measured	Holdway (1992)
												168 (+ 168 h post exposure)	1015 (LC_{50}) (900–1190)	3.8 (LC_{50}) (3.3–4.40)		
											Growth	168	1780 (LOEC) 920 (NOEC)	6.6 (LOEC) 3.4 (NOEC)		
												168 (+ 168 h post exposure)	455 (LOEC) < 455 (NOEC)	1.7 (LOEC) < 1.7 (NOEC)		
	Uranium	Larvae (6 d)	Flow-through	Buffalo Billabong water	30 ± 0.1	6.6 ± 0.2	38 (36–40)	5.1	3.2	5.8 (D)	Survival	96	1790 (LC_{50}) (1385–2100)	6.6 (LC_{50}) (5.1–7.8)	Measured	Holdway (1992)
											Growth	96	1240 (LOEC) 640 (NOEC)	4.6 (LOEC) 2.4 (NOEC)		
	Uranium	Juvenile (40 d)	Flow-through	Buffalo Billabong water	30 ± 0.1	6.3 ± 0.2	38 (36–40)	5.1	3.2	5.8 (D)	Survival	96	3750 (LC_{50}) (2580–4925)	13.9 (LC_{50}) (9.5–18.2)	Measured	Holdway (1992)
												168	3070 (LC_{50}) (2580–3590)	11.4 (LC_{50}) (9.5–13.3)		
											Growth	168 (+ 168 h post exposure)	1640 (LC_{50}) (1120–2565)	6.1 (LC_{50}) (4.1–9.5)		
												168	4930 (LOEC) 2580 (NOEC)	18.2 (LOEC) 9.5 (NOEC)		
												168 (+ 168 h post exposure)	2580 (LOEC) 1240 (NOEC)	9.5 (LOEC) 4.6 (NOEC)		

Appendix A Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	pH	Conductivity (µS cm^{-1})	Hardness (mg $CaCO_3$ L^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})	Organic carbon[b] (mg L^{-1})	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})[i]	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Purple-spotted gudgeon (*Mogurnda mogurnda*)	Uranium	Juvenile (70 d)	Flow-through	Buffalo Billabong water	30 ± 0.1	6.6 ± 0.2	38 (36–40)	5.1	3.2	5.8 (D)	Survival	96	3750 (LC_{50}) (2580–4925)	13.9 (LC_{50}) (9.5–18.2)	Measured	Holdway (1992)
												168	3750 (LC_{50}) (2580–4925)	13.9 (LC_{50}) (9.5–18.2)		
												168 (+ 168 h post exposure)	3078 (LC_{50}) (2580–3590)	11.4 (LC_{50}) (9.5–13.3)		
	Uranium	Sac-fry (1 d)	Static-renewal	Synthetic Magela Creek water	27 ± 1	6.0 ± 0.1	23 (22–24)	3.9 (3.8–4.0)	4.1 (4.0–4.2)	< 0.2 (D)	Survival	96	1270 (BEC_{10}) 1300 (MDEC) 1570 (LC_{50}) (1510–1630)	4.7 (BEC_{10}) 4.8 (MDEC) 5.8 (LC_{50}) (5.6–6.0)	Measured	Markich & Camilleri (This study)
Eel-tailed catfish (*Porochilus rendahli*)	Copper	Adult	Flow-through	Synthetic Gulungul Creek water	27.0 ± 0.5	6.0 ± 0.1	NR	25–30	5	< 0.5 (T)	Survival	48	210 (LC_{50}) (160–250)	3.3 (LC_{50}) (2.5–3.9)	Measured	Williams et al (1991)
												72	85 (LC_{50}) (17–125)	1.34 (LC_{50}) (0.27–2.0)		
Blue eye (*Pseudomugil tenellus*)	Copper	Adult	Static	Ja Ja Billabong water	25 ± 1	6.0 ± 0.4	64[n] (47–81)	8[n] (6–10)	4[n] (2–6)	11[n] (7.3–14.7) (D) 14[n] (9.5–18.5) (T)	Survival	96	120 (LC_{50})	1.89 (LC_{50})	Nominal	R Bolas & J Skidmore (Pers comm)
	Uranium	Juvenile	Static-renewal	Magela Creek water	27	6.6 ± 0.1	16 (15–17)	4.8 (4.6–5.0)	3.3 (3.0–3.6)	NR	Survival	48	940 (LC_{50}) (640–1230)	3.5 (LC_{50}) (2.4–4.6)	Measured	Bywater et al (1991)
												72	830 (LC_{50}) (570–1070)	3.1 (LC_{50}) (2.1–4.0)		
												96	830 (LC_{50}) (570–1070)	3.1 (LC_{50}) (2.1–4.0)		

[a] All numerical values represent mean values, or their range, with the 95% confidence interval (CI) in parentheses (where reported). Means shown with ± values were regulated within the reported limits. NR: not reported. Concentrations of dissolved oxygen were maintained at near-saturation for all tests, where this parameter was measured. Uranium concentration is expressed as uranyl (*ie* UO_2); this was derived by multiplying the U concentration by 1.14.

[b] T, total; D, dissolved.

[c] BEC_{10}, 10% bounded-effect concentration (Hoekstra & van Ewijk 1993), an analogous statistical measure of the no-observed effect concentration (NOEC).

[d] MDEC, minimum detectable effect concentration (Ahsanullah & Williams 1991), an analogous statistical measure of the lowest-observed effect concentration (LOEC).

[e] EC_{50}, median effect concentration.

[f] Estimated from established protocols (*ie* Holdway & Wiecek 1988, Allison et al 1991, Holdway 1992, Hyne et al 1996).

[g] Not reported.

[h] LOEC, Lowest-observed effect concentration.

[i] Estimated from the mean physico-chemistry of Sydney tap water (Markich unpub).

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

[k] The frequency of valve adductions (*ie* movements) was the measured behavioural characteristic.

[l] The duration of valve opening was the measured behavioural characteristic.

[m] NOEC, No-observed effect concentration.

[n] Estimated from the mean physico-chemistry of Ja Ja Billabong during the Dry season of 1982 (NTDTW 1983).

Appendix B Summary of metal toxicity data for Australian tropical marine biota[a]

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Algae												
Diatom (*Nitzschia closterium*)	Chromium (VI)	Log phase growth (< 7 d)	Static	Filtered sea water	27 ± 1	31	Population growth	72	3500 (EC_{50})[b] 1000 (MATC)[c]	67 (EC_{50})[b] 19 (MATC)[c]	Nominal	Florence et al (1994)
	Lead	Log phase growth (< 7 d)	Static	Filtered sea water	29	31	Population growth	72	> 365 (NOEC)[d]	> 1.8 (NOEC)[d]	Nominal	J Stauber (Pers comm)
	Nickel	Log phase growth (< 7 d)	Static	Filtered sea water	29 ± 1	31	Population growth	72	> 500 (NOEC)	> 8.5 (NOEC)	Nominal	Florence et al (1994)
	Zinc	Log phase growth (< 7 d)	Static	Filtered sea water	29 ± 1	31	Population growth	72	197 (EC_{50}) 180 (LOEC)[e] 150 (NOEC)	3.0 (EC_{50}) 2.7 (LOEC)[e] 2.3 (NOEC)	Nominal	J Stauber (Pers comm)
Cnidaria												
Coral (*Goniastrea aspera*)	Copper	Embryo	Static	Filtered sea water	26	35	Fertilisation	4	26 (EC_{50})	0.41 (EC_{50})	Nominal	Reichelt & Harrison (1995)
Mollusca												
Gastropod (*Polinices ineci*)	Copper	Adult	Static-renewal	Sea water	20	32	Survival	96	1170 (LC_{50})[f]	18 (LC_{50})[f]	Nominal	Chapman et al (1985)
Gastropod (*Polinices sordidus*)	Copper	Adult	Static-renewal	Sea water	20	32	Survival	96	770 (LC_{50})	12 (LC_{50})	Nominal	Hughes et al (1987)
Crustacea												
Crab (*Carybdis feriatus*)	Cadmium	Stage 1 zoeae larvae	Static	Filtered sea water	26 ± 1	32	Survival	48	250 (LC_{50}) (210–300)	2.2 (LC_{50}) (1.9–2.7)	Nominal	Greenwood & Fielder (1983)
	Zinc	Stage 1 zoeae larvae	Static	Filtered sea water	26 ± 1	32	Survival	48	960 (LC_{50}) (410–2280)	14.7 (LC_{50}) (6.3–35)	Nominal	Greenwood & Fielder (1983)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Banana prawn (*Penaeus merguiensis*)	Cadmium	Juvenile (28 d)	Static-renewal	Filtered sea water	20	20	Survival	96	1100 (LC_{50}) (520–2310)	9.8 (LC_{50}) (4.6–21)	Nominal	Denton & Burdon-Jones (1982)
	Cadmium	Juvenile (28 d)	Static-renewal	Filtered sea water	20	36	Survival	96	1850 (LC_{50}) (1060–3240)	17 (LC_{50}) (9.4–28.8)	Nominal	Denton & Burdon-Jones (1982)
	Cadmium	Juvenile (28 d)	Static-renewal	Filtered sea water	30	20	Survival	96	650 (LC_{50}) (420–1010)	5.8 (LC_{50}) (3.7–9.0)	Nominal	Denton & Burdon-Jones (1982)
	Cadmium	Juvenile (28 d)	Static-renewal	Filtered sea water	30	36	Survival	96	1200 (LC_{50}) (500–2600)	11 (LC_{50}) (4.5–23)	Nominal	Denton & Burdon-Jones (1982)
	Cadmium	Juvenile (28 d)	Static-renewal	Filtered sea water	35	20	Survival	96	370 (LC_{50}) (190–700)	3.3 (LC_{50}) (1.7–6.2)	Nominal	Denton & Burdon-Jones (1982)
	Cadmium	Juvenile (28 d)	Static-renewal	Filtered sea water	35	36	Survival	96	150 (LC_{50}) (60–340)	1.3 (LC_{50}) (0.53–3.0)	Nominal	Denton & Burdon-Jones (1982)
	Copper	Juvenile (28 d)	Static-renewal	Filtered sea water	20	20	Survival	96	720 (LC_{50}) (240–2160)	11 (LC_{50}) (3.8–34)	Nominal	Denton & Burdon-Jones (1982)
	Copper	Juvenile (28 d)	Static-renewal	Filtered sea water	20	36	Survival	96	6100 (LC_{50}) (2640–14600)	98 (LC_{50}) (42–230)	Nominal	Denton & Burdon-Jones (1982)
	Copper	Juvenile (28 d)	Static-renewal	Filtered sea water and fresh water	27	20	Survival	96	380 (LC_{50}) (210–680)	6.0 (LC_{50}) (3.3–11)	Measured	Ahsanullah & Ying (1995)
							Growth	336	50 (NOEC)	0.79 (NOEC)		
	Copper	Juvenile (28 d)	Static-renewal	Filtered sea water	30	20	Survival	96	530 (LC_{50}) (250–1110)	8.3 (LC_{50}) (3.9–18)	Nominal	Denton & Burdon-Jones (1982)
	Copper	Juvenile (28 d)	Static-renewal	Filtered sea water	30	36	Survival	96	900 (LC_{50}) (500–1620)	14 (LC_{50}) (7.9–26)	Nominal	Denton & Burdon-Jones (1982)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Banana prawn (*Penaeus merguiensis*)	Copper	Juvenile (28 d)	Static-renewal	Filtered sea water	35	20	Survival	96	210 (LC_{50}) (140–320)	3.3 (LC_{50}) (2.2–5.0)	Nominal	Denton & Burdon-Jones (1982)
	Copper	Juvenile (28 d)	Static-renewal	Filtered sea water	35	36	Survival	96	350 (LC_{50}) (180–670)	5.5 (LC_{50}) (2.8–11)	Nominal	Denton & Burdon-Jones (1982)
	Lead	Juvenile (28 d)	Static-renewal	Filtered sea water	20	20	Survival	96	51000 (LC_{50}) (32200–80600)	246 (LC_{50}) (155–389)	Nominal	Denton & Burdon-Jones (1982)
	Lead	Juvenile (28 d)	Static-renewal	Filtered sea water	20	36	Survival	96	195000 (LC_{50}) (135000–280000)	941 (LC_{50}) (651–1350)	Nominal	Denton & Burdon-Jones (1982)
	Lead	Juvenile (28 d)	Static-renewal	Filtered sea water	30	20	Survival	96	36500 (LC_{50}) (19200–69400)	176 (LC_{50}) (93–335)	Nominal	Denton & Burdon-Jones (1982)
	Lead	Juvenile (28 d)	Static-renewal	Filtered sea water	30	36	Survival	96	80000 (LC_{50}) (45700–140000)	386 (LC_{50}) (221–676)	Nominal	Denton & Burdon-Jones (1982)
	Lead	Juvenile (28 d)	Static-renewal	Filtered sea water	35	20	Survival	96	30000 (LC_{50}) (14300–63000)	145 (LC_{50}) (69–304)	Nominal	Denton & Burdon-Jones (1982)
	Lead	Juvenile (28 d)	Static-renewal	Filtered sea water	35	36	Survival	96	31000 (LC_{50}) (12400–77500)	150 (LC_{50}) (60–374)	Nominal	Denton & Burdon-Jones (1982)
	Mercury	Juvenile (28 d)	Static-renewal	Filtered sea water	20	20	Survival	96	130 (LC_{50}) (70–240)	0.65 (LC_{50}) (0.35–1.2)	Nominal	Denton & Burdon-Jones (1982)
	Mercury	Juvenile (28 d)	Static-renewal	Filtered sea water	20	36	Survival	96	290 (LC_{50}) (140–570)	1.5 (LC_{50}) (0.70–2.8)	Nominal	Denton & Burdon-Jones (1982)
	Mercury	Juvenile (28 d)	Static-renewal	Filtered sea water	30	20	Survival	96	160 (LC_{50}) (100–260)	0.80 (LC_{50}) (0.50–1.3)	Nominal	Denton & Burdon-Jones (1982)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Banana prawn (*Penaeus merguiensis*)	Mercury	Juvenile (28 d)	Static-renewal	Filtered sea water	30	36	Survival	96	70 (LC_{50}) (40–110)	0.35 (LC_{50}) (0.20–0.55)	Nominal	Denton & Burdon-Jones (1982)
	Mercury	Juvenile (28 d)	Static-renewal	Filtered sea water	35	20	Survival	96	30 (LC_{50}) (10–60)	0.15 (LC_{50}) (0.05–0.30)	Nominal	Denton & Burdon-Jones (1982)
	Mercury	Juvenile (28 d)	Static-renewal	Filtered sea water	35	36	Survival	96	30 (LC_{50}) (10–70)	0.15 (LC_{50}) (0.05–0.35)	Nominal	Denton & Burdon-Jones (1982)
	Nickel	Juvenile (28 d)	Static-renewal	Filtered sea water	20	20	Survival	96	21000 (LC_{50}) (10800–41000)	358 (LC_{50}) (184–698)	Nominal	Denton & Burdon-Jones (1982)
	Nickel	Juvenile (28 d)	Static-renewal	Filtered sea water	20	36	Survival	96	21000 (LC_{50})	358 (LC_{50})	Nominal	Denton & Burdon-Jones (1982)
	Nickel	Juvenile (28 d)	Static-renewal	Filtered sea water	30	20	Survival	96	6600 (LC_{50}) (2750–15800)	112 (LC_{50}) (47–269)	Nominal	Denton & Burdon-Jones (1982)
	Nickel	Juvenile (28 d)	Static-renewal	Filtered sea water	30	36	Survival	96	3550 (LC_{50}) (1760–7100)	60.5 (LC_{50}) (30–121)	Nominal	Denton & Burdon-Jones (1982)
	Nickel	Juvenile (28 d)	Static-renewal	Filtered sea water	35	20	Survival	96	6900 (LC_{50}) (4180–11400)	118 (LC_{50}) (71–194)	Nominal	Denton & Burdon-Jones (1982)
	Nickel	Juvenile (28 d)	Static-renewal	Filtered sea water	35	36	Survival	96	6900 (LC_{50}) (4180–11400)	118 (LC_{50}) (71–194)	Nominal	Denton & Burdon-Jones (1982)
	Zinc	Juvenile (28 d)	Static-renewal	Filtered sea water	20	20	Survival	96	4800 (LC_{50}) (2200–10700)	73 (LC_{50}) (34–164)	Nominal	Denton & Burdon-Jones (1982)
	Zinc	Juvenile (28 d)	Static-renewal	Filtered sea water	20	36	Survival	96	6600 (LC_{50})	101 (LC_{50})	Nominal	Denton & Burdon-Jones (1982)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Banana prawn (*Penaeus merguiensis*)	Zinc	Juvenile (28 d)	Static-renewal	Filtered sea water	30	20	Survival	96	500 (LC_{50}) (300–840)	7.7 (LC_{50}) (4.6–13)	Nominal	Denton & Burdon-Jones (1982)
	Zinc	Juvenile (28 d)	Static-renewal	Filtered sea water	30	36	Survival	96	1200 (LC_{50}) (450–2640)	18 (LC_{50}) (6.9–40)	Nominal	Denton & Burdon-Jones (1982)
	Zinc	Juvenile (28 d)	Static-renewal	Filtered sea water	35	20	Survival	96	500 (LC_{50}) (240–1100)	7.7 (LC_{50}) (3.7–17)	Nominal	Denton & Burdon-Jones (1982)
	Zinc	Juvenile (28 d)	Static-renewal	Filtered sea water	35	36	Survival	96	370 (LC_{50}) (190–740)	5.7 (LC_{50}) (2.9–11)	Nominal	Denton & Burdon-Jones (1982)
Leader prawn (*Penaeus monodon*)	Copper	Juvenile (28 d)	Flow-through	Filtered sea water and fresh water	27	20	Survival	96	> 2500 (LC_{50})	> 39 (LC_{50})	Measured	Ahsanullah & Ying (1995)
							Growth	336	> 200 (NOEC)	> 3.2 (NOEC)		
Crab (*Portunus pelagicus*)	Cadmium	Stage 1 zoeae larvae	Static	Filtered sea water	26 ± 1	32	Survival	48	380 (LC_{50}) (170–850)	3.4 (LC_{50}) (1.5–7.6)	Nominal	Greenwood & Fielder (1983)
	Chromium (VI)	Stage 1 zoeae larvae	Static-renewal	Filtered sea water	26 ± 1	33	Moult inhibition	48	320 (MATC)	6.2 (MATC)	Nominal	Mortimer & Miller (1994)
							Survival		1850 (LC_{50}) (800–3900)	36 (LC_{50}) (15–75)		
	Chromium (VI)	Stage 3 zoeae larvae	Static-renewal	Filtered sea water	26 ± 1	33	Moult inhibition	48	1700–5500 (MATC)	33–106 (MATC)	Nominal	Mortimer & Miller (1994)
							Survival		6800 (LC_{50}) (1700–11900)	131 (LC_{50}) (33–229)		
	Chromium (VI)	Megalopa larvae	Static-renewal	Filtered sea water	26 ± 1	25	Moult inhibition	120	1700 (MATC)	33 (MATC)	Nominal	Mortimer & Miller (1994)
							Survival		3800 (LC_{50}) (1500–9300)	73 (LC_{50}) (29–179)		
	Chromium (VI)	Stage 3–6 juvenile	Static-renewal	Filtered sea water	26 ± 1	25	Growth	960–1008 (40–42 d)	550 (MATC)	11 (MATC)	Nominal	Mortimer & Miller (1994)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Crab (*Portunus pelagicus*)	Copper	Stage 3 zoeae larvae	Static-renewal	Filtered sea water	26 ± 1	33	Survival	24	110 (LC_{50}) (80–140)	1.7 (LC_{50}) (1.3–2.2)	Nominal	Mortimer & Miller (1994)
								48	50 (LC_{50}) (40–70)	0.79 (LC_{50}) (0.63–1.1)		
							Moult inhibition	48	< 10 (MATC)	< 0.16 (MATC)		
	Nickel	Stage 3 zoeae larvae	Static-renewal	Filtered sea water	26 ± 1	33	Survival	48	1130 (LC_{50}) (100–6800)	22 (LC_{50}) (1.9–131)	Nominal	Mortimer & Miller (1994)
	Nickel	Megalopa larvae	Static-renewal	Filtered sea water	26 ± 1	25	Moult inhibition	120	32 (MATC)	0.55 (MATC)	Nominal	Mortimer & Miller (1994)
							Survival		1300 (LC_{50}) (400–4800)	22 (LC_{50}) (6.8–82)		
	Nickel	Stage 1–6 juvenile	Static-renewal	Filtered sea water	26 ± 1	25	Growth	960–1008 (40–42 d)	32 (MATC)	0.55 (MATC)	Nominal	Mortimer & Miller (1994)
	Zinc	Stage 1 zoeae larvae	Static	Filtered sea water	26 ± 1	32	Survival	48	650 (LC_{50}) (440–950)	9.9 (LC_{50}) (6.7–15)	Nominal	Greenwood & Fielder (1983)
Crab (*Portunus sanguinolentus*)	Cadmium	Stage 1 zoeae larvae	Static	Filtered sea water	26 ± 1	32	Survival	48	250 (LC_{50}) (220–290)	2.2 (LC_{50}) (2.0–2.6)	Nominal	Greenwood & Fielder (1983)
	Zinc	Stage 1 zoeae larvae	Static	Filtered sea water	26 ± 1	32	Survival	48	620 (LC_{50}) (530–730)	9.5 (LC_{50}) (8.1–11)	Nominal	Greenwood & Fielder (1983)
Chordata												
Diamond-scaled mullet (*Liza vaigiensis*)	Cadmium	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	5250 (LC_{50}) (3980–6930)	47 (LC_{50}) (35–62)	Nominal	Denton & Burdon-Jones (1986)
	Cadmium	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	7800 (LC_{50}) (6700–9100)	69 (LC_{50}) (60–81)	Nominal	Denton & Burdon-Jones (1986)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (μg L^{-1})	Water concentration (μmol L^{-1})	Concentration (measured or nominal)	Reference
Diamond-scaled mullet (*Liza vaigiensis*)	Copper	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	2650 (LC_{50}) (1610–4370)	42 (LC_{50}) (25–69)	Nominal	Denton & Burdon-Jones (1986)
	Copper	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	2550 (LC_{50}) (1700–3830)	40 (LC_{50}) (27–60)	Nominal	Denton & Burdon-Jones (1986)
	Lead	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	98000 (LC_{50}) (79700–121000)	473 (LC_{50}) (385–584)	Nominal	Denton & Burdon-Jones (1986)
	Lead	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	190000 (LC_{50}) (179000–201000)	917 (LC_{50}) (864–970)	Nominal	Denton & Burdon-Jones (1986)
	Mercury	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	330 (LC_{50}) (240–460)	1.7 (LC_{50}) (1.2–2.3)	Nominal	Denton & Burdon-Jones (1986)
	Mercury	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	380 (LC_{50}) (260–550)	1.9 (LC_{50}) (1.3–2.7)	Nominal	Denton & Burdon-Jones (1986)
	Nickel	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	40000 (LC_{50}) (33800–47400)	681 (LC_{50}) (576–807)	Nominal	Denton & Burdon-Jones (1986)
	Nickel	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	55500 (LC_{50}) (45900–67200)	945 (LC_{50}) (782–1150)	Nominal	Denton & Burdon-Jones (1986)
	Zinc	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	12500 (LC_{50}) (9760–16000)	191 (LC_{50}) (149–245)	Nominal	Denton & Burdon-Jones (1986)
	Zinc	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	18500 (LC_{50}) (15700–21800)	283 (LC_{50}) (240–333)	Nominal	Denton & Burdon-Jones (1986)
Glass perch (*Priopidichthys marianus*)	Cadmium	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	13500 (LC_{50}) (5630–32000)	120 (LC_{50}) (50.0–285)	Nominal	Denton & Burdon-Jones (1986)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Glass perch (*Priopidichthys marianus*)	Cadmium	Adult	Static-renewal	Filtered sea water	20	36	Survival	96	45000 (LC_{50}) (38100–53100)	400 (LC_{50}) (339–472)	Nominal	Denton & Burdon-Jones (1986)
	Cadmium	Juvenile	Static-renewal	Filtered sea water	30	20	Survival	96	21000 (LC_{50}) (16900–26000)	187 (LC_{50}) (150–231	Nominal	Denton & Burdon-Jones (1986)
	Cadmium	Juvenile	Static-renewal	Filtered sea water	30	36	Survival	96	18000 (LC_{50}) (13200–24500)	160 (LC_{50}) (117–218)	Nominal	Denton & Burdon-Jones (1986)
	Copper	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	6000 (LC_{50}) (4280–8400)	94 (LC_{50}) (67–132)	Nominal	Denton & Burdon-Jones (1986)
	Copper	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	2100 (LC_{50}) (1400–3150)	33 (LC_{50}) (22–50)	Nominal	Denton & Burdon-Jones (1986)
	Copper	Adult	Static-renewal	Filtered sea water	20	36	Survival	96	2550 (LC_{50}) (2010–3240)	40 (LC_{50}) (32–51)	Nominal	Denton & Burdon-Jones (1986)
	Copper	Juvenile	Static-renewal	Filtered sea water	30	20	Survival	96	4400 (LC_{50}) (2490–7790)	69 (LC_{50}) (39–122)	Nominal	Denton & Burdon-Jones (1986)
	Copper	Juvenile	Static-renewal	Filtered sea water	30	36	Survival	96	3000 (LC_{50}) (2240–4020)	47 (LC_{50}) (35–63)	Nominal	Denton & Burdon-Jones (1986)
	Lead	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	80000 (LC_{50}) (68100–94000)	386 (LC_{50}) (329–454)	Nominal	Denton & Burdon-Jones (1986)
	Lead	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	160000 (LC_{50}) (140000–180000)	772 (LC_{50}) (676–869)	Nominal	Denton & Burdon-Jones (1986)
	Lead	Adult	Static-renewal	Filtered sea water	20	36	Survival	96	183000 (LC_{50}) (146000–229000)	883 (LC_{50}) (705–1100)	Nominal	Denton & Burdon-Jones (1986)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (μg L^{-1})	Water concentration (μmol L^{-1})	Concentration (measured or nominal)	Reference
Glass perch (*Priopidichthys marianus*)	Lead	Juvenile	Static-renewal	Filtered sea water	30	20	Survival	96	110000 (LC_{50}) (75000–140000)	531 (LC_{50}) (362–676)	Nominal	Denton & Burdon-Jones (1986)
	Lead	Juvenile	Static-renewal	Filtered sea water	30	36	Survival	96	160000 (LC_{50}) (140000–180000)	772 (LC_{50}) (676–869)	Nominal	Denton & Burdon-Jones (1986)
	Mercury	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	650 (LC_{50}) (430–990)	3.2 (LC_{50}) (2.1–4.9)	Nominal	Denton & Burdon-Jones (1986)
	Mercury	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	420 (LC_{50}) (290–600)	2.1 (LC_{50}) (1.5–3.0)	Nominal	Denton & Burdon-Jones (1986)
	Mercury	Adult	Static-renewal	Filtered sea water	20	36	Survival	96	660 (LC_{50}) (320–1000)	3.3 (LC_{50}) (1.60–4.99)	Nominal	Denton & Burdon-Jones (1986)
	Mercury	Juvenile	Static-renewal	Filtered sea water	30	20	Survival	96	500 (LC_{50}) (330–750)	2.5 (LC_{50}) (1.7–3.7)	Nominal	Denton & Burdon-Jones (1986)
	Mercury	Juvenile	Static-renewal	Filtered sea water	30	36	Survival	96	350 (LC_{50}) (260–470)	1.7 (LC_{50}) (1.3–2.3)	Nominal	Denton & Burdon-Jones (1986)
	Nickel	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	30000 (LC_{50}) (23100–39000)	511 (LC_{50}) (393–664)	Nominal	Denton & Burdon-Jones (1986)
	Nickel	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	47500 (LC_{50}) (40900–55100)	809 (LC_{50}) (697–939)	Nominal	Denton & Burdon-Jones (1986)
	Nickel	Adult	Static-renewal	Filtered sea water	20	36	Survival	96	100000 (LC_{50}) (80000–125000)	1703 (LC_{50}) (1363–2129)	Nominal	Denton & Burdon-Jones (1986)
	Nickel	Juvenile	Static-renewal	Filtered sea water	30	20	Survival	96	42000 (LC_{50}) (33600–52500)	715 (LC_{50}) (572–894)	Nominal	Denton & Burdon-Jones (1986)

Appendix B Cont'd

Species	Metal	Life stage	Mode of exposure	Water type	Temperature (°C)	Salinity (‰)	Test endpoint	Duration of exposure (h)	Water concentration (µg L^{-1})	Water concentration (µmol L^{-1})	Concentration (measured or nominal)	Reference
Glass perch (*Priopidichthys marianus*)	Nickel	Juvenile	Static-renewal	Filtered sea water	30	36	Survival	96	44500 (LC_{50}) (36500–54300)	758 (LC_{50}) (622–925)	Nominal	Denton & Burdon-Jones (1986)
	Zinc	Juvenile	Static-renewal	Filtered sea water	20	20	Survival	96	16000 (LC_{50}) (11400–22400)	245 (LC_{50}) (174–343)	Nominal	Denton & Burdon-Jones (1986)
	Zinc	Juvenile	Static-renewal	Filtered sea water	20	36	Survival	96	17500 (LC_{50}) (14800–20700)	268 (LC_{50}) (226–317)	Nominal	Denton & Burdon-Jones (1986)
	Zinc	Adult	Static-renewal	Filtered sea water	20	36	Survival	96	23800 (LC_{50}) (19400–29200)	364 (LC_{50}) (297–447)	Nominal	Denton & Burdon-Jones (1986)
	Zinc	Juvenile	Static-renewal	Filtered sea water	30	20	Survival	96	17000 (LC_{50}) (14500–20000)	260 (LC_{50}) (222–306)	Nominal	Denton & Burdon-Jones (1986)
	Zinc	Juvenile	Static-renewal	Filtered sea water	30	36	Survival	96	19200 (LC_{50}) (14600–25200)	294 (LC_{50}) (223–385)	Nominal	Denton & Burdon-Jones (1986)

[a] All numerical values represent mean values, or their range, with the 95% confidence interval (CI) in parentheses (where reported). Means shown with ± values were regulated within the reported limits. Concentrations of dissolved oxygen were maintained at near-saturation for all tests, where this parameter was measured. The pH of sea water used in all tests ranged from 7.8–8.3 and the photoperiod was usually 12 h light: 12 h dark.

[b] EC_{50}, median effect concentration.

[c] MATC, Maximum acceptable toxicant concentration (defined as the geometric mean of the highest no-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC))

[d] NOEC, No-observed effect concentration.

[e] LOEC, Lowest-observed effect concentration.

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

Appendix C Test protocols

C.1 Green hydra (*H. viridissima*) population growth test

C.1.1 Objective

The objective of the test was to determine the concentration of a specified chemical that shows:

(a) no effect (ie concentration showing no statistical difference (P ≤0.05) between exposed and unexposed or control specimens) (measured using the 10% bounded effect concentration, BEC_{10}; see Hoekstra & Van Ewijk 1993);

(b) a lowest effect (ie concentration showing the smallest statistical difference (P ≤0.05) between exposed and unexposed or control specimens) (measured as the minimum detectable effect concentration, MDEC; see Ahsanullah & Williams 1991), and

(c) a median effect (concentration showing a 50% decline) (measured as the EC_{50}) on the population growth of *Hydra viridissima* (green hydra) over 96 h.

C.1.2 Principle of the test

Asexually reproducing (budding) test hydra are exposed to a range of chemical concentrations for 96 h. Observations of any changes to the hydra population (ie changes in the number of intact hydroids; one hydroid equals one animal plus any attached buds) are recorded at 24 h intervals. The method is based on the Hydra Population Growth Test described by Hyne et al (1996).

C.1.3 Test organism

The species is *Hydra viridissima* (Cnidaria, Hydrozoa). *H. viridissima* is referred to as 'green' hydra because of its green colouration resulting from the presence of a symbiotic green alga in the gastrodermal cells of the animal. Although the precise distribution of this species has not been mapped, it has been found in a variety of aquatic habitats in northern Australia. Test hydra were obtained from laboratory cultures as described in Appendix F. Test hydra are selected as hydra that were budding with one bud that is just showing signs of becoming tentacled. Asexual budding is a characteristic of hydra in optimal environmental conditions. Hydra selected for testing must be free of overt disease and gross morphological deformity (ie show no signs of clubbing or contraction).

C.1.4 Synthetic water

The test water is an artificial or 'synthetic' water that simulates the inorganic composition of Magela Creek water during the wet season (see below). Magela Creek water is very soft, slightly acidic and has a low buffering and complexation capacity. These qualities are predicted to maximise the toxic response of an organism, and hence, provide the greatest probability of detriment to organisms exposed to metals. The ionic composition of Magela Creek water is representative of sandy braided streams throughout much of wet/dry tropics (C leGras pers comm). The synthetic water is prepared by adding analytical grade reagents to deionised (DI) water (< 1µS cm^{-1}) in acid-washed polyethylene containers, as close as is practical to the start of the test. The pH of the test water is adjusted to the required level (in this case 6.0 ± 0.15) with dilute acid and/or base. The test water should be stored in sealed polyethylene containers and refrigerated (4°C) until use.

C.1.5 Stock solutions

Analytical grade reagents are used to prepare stock solutions. A stock solution of the appropriate chemical is prepared in a clean, inert container and refrigerated (4°C). The source of the stock solution (eg date of preparation, by whom), is described on an information sheet.

C.1.6 Test solutions

Test solutions are prepared by serially diluting a stock solution with pH-adjusted synthetic water. The pH is then re-adjusted if necessary. Test solution concentrations are determined from the results of range-finding studies. Test solutions are prepared in bulk at the start of a test in 5 L polyethylene screw-topped containers and refrigerated (4°C) until required. Alternatively, test solutions are prepared daily if it is established that the toxicity of the test solution varies appreciably when stored during the period of the test.

C.1.7 Apparatus and test equipment

All materials that come into contact with (i) any liquid into which the hydra are placed or (ii) the hydra themselves, should be chemically inert.

Mean nominal composition of the synthetic water

Physico-chemical parameter	Background water[a]
pH	6.0 ± 0.15[a]
Temperature (°C)	27 ± 1[a]
Na (mg L^{-1})	1.00
K (mg L^{-1})	0.37
Ca (mg L^{-1})	0.45
Mg (mg L^{-1})	0.60
Cl (mg L^{-1})	2.32
SO_4 (mg L^{-1})	3.12
HCO_3 (mg L^{-1})	2.63
NO_3 (µg L^{-1})	0.07
Fe (µg L^{-1})	100
Al (µg L^{-1})	70
Mn (µg L^{-1})	9.7
U (µg L^{-1})	0.10
Cu (µg L^{-1})	0.70
Zn (µg L^{-1})	0.70
Pb (µg L^{-1})	0.12

[a] The pH and temperature were tightly regulated as described in the text.

(a) Container preparation

All containers (ie vials, bottles, Petri dishes and lids etc) and Pasteur pipettes used in any part of the test are prepared in the following manner:

- Undergo a dish washer (Gallay Laboratory 999) cycle, containing detergent (Gallay Clean A phosphate free) and acid (double strength), using reverse osmosis (RO) grade water for two rinse cycles;
- rinse with DI water (< 1 μS cm-1); and
- allow to air dry.

OR

- immerse in a 1–3% detergent solution (eg Decon Neutracon) for up to 24 h;
- scrub to remove extraneous material, then rinse thoroughly in tap water;
- immediately immerse in a 5% HNO_3 solution for up to 24 h;
- thoroughly rinse at least 3 times with DI water (< 1 μS cm^{-1}); and
- allow to air dry.

Immediately before use the containers should be rinsed with pH-adjusted synthetic water. Other equipment should be rinsed thoroughly with DI water (< 1 μS cm^{-1}) before use.

(b) Temperature control

Tests were conducted at 27 ± 1°C using a constant temperature incubator. The temperature of the test containers was maintained at 27 ± 1°C (eg by the use of warming trays set at 27°C, and placed on the microscope bench) when they were removed from the incubator for observation.

(c) Photoperiod control

Tests were conducted with a 12 h light:dark photoperiod, where the mid-point coincides with solar midday. Light intensity should be typical for normal laboratory working conditions (ie 10–50 μE m^{-2} s^{-1} Photosynthetic Active Radiation).

(d) Equipment

- seven 5 L polyethylene containers
- refrigerator for storage of test and stock solutions
- twenty-one 45 mL disposable plastic vials with screw-capped lids
- twenty-one 90 mm diameter disposable plastic Petri dishes with lids
- fourteen 100 mL disposable plastic vials with screw-capped lids (for water parameter measurement)
- maximum-minimum thermometers
- calibrated mercury thermometer
- pH meter, pH probe, and pH buffer solutions of 6.87 and 4.01
- conductivity meter and probe
- dissolved oxygen meter fitted with a micro-oxygen electrode
- binocular dissecting microscope with bright field/dark field illumination
- automatic 0–50 mL dispenser
- clear plastic trays capable of holding 21 Petri dishes, with position numbers 1 to 21 marked

- laboratory warming trays, set at 27°C, capable of accommodating the clear plastic trays
- random number generator
- two Perspex trays, each of such a size to hold 10 vials
- Pasteur pipettes, with internal tip diameter > 2 mm

C.1.8 Test environment

The preparation and storage of test solutions, culturing of hydra to be used in the tests, and manipulation and testing should be carried out in premises free from harmful vapours and dusts, and any undue disturbance. All workers involved in any part of the test should wash hands and arms thoroughly with fragrance-free soap and rinse well with tap water before commencing any part of the test procedure.

C.1.9 Data recording

Test animals are observed and data are recorded at 24 h intervals after the commencement of the test (when t = 0 h). Observations made at the end of the first 24 h period are designated as Day 1 observations; at the end of the second 24 h period, Day 2 observations etc. Water parameters are measured and adjusted (where appropriate) and recorded at the beginning and end of each 24 h period, and are designated as Fresh Water Day 1, 24 h-old Water Day 1, respectively, and so forth during the test.

C.1.10 Test procedure

Day 1

1 Prepare the test solutions (as outlined in Section C.1.6) and leave at room temperature.

2 Isolate approximately 220 suitable hydra in synthetic water in 3 petri dishes and leave at room temperature. A 'suitable test hydra' is a hydra with one bud. The bud must not be fully developed (ie tentacles are present only as 'bumps', and the bud must not appear ready to detach from the main stem of the hydroid).

3 Dispense 30 mL aliquots of each test concentration (normally 7) into 3 appropriately labelled replicate Petri dishes (ie 3 x 30 mL for each test solution), and arrange in three replicate groups on clear plastic trays (eg Control replicate 1 to X $\mu g\ L^{-1}$ on Tray 1).

4 Using a microscope and Pasteur pipette, pick out one hydra from the isolated stock and place into Control replicate 1.

5 Repeat for remaining test concentrations of replicate 1, working up in concentration, and ending with the highest concentration.

6 Discard the used pipette and select a new one.

7 Repeat steps 4–6 until all test dishes for that replicate group contain 10 hydra.

8 Observe each dish under the microscope to ensure that there are 10 hydra in each dish, and replace any hydra that are damaged in any way (eg all buds must be attached). If not, replace immediately with 'suitable test hydra' using a new pipette.

9 Repeat steps 4–8 for the remaining two replicate groups.

More than one person can distribute test hydra simultaneously, with the distribution appropriately split into replicate groups.

10 Cover the dishes and place them in the random order for that day (see below), in the positions 1 to 21.

11 Place trays in the incubator.

Completion of this stage constitutes the start of the test (time = 0 h).

Note: Whenever test dishes are removed from the incubator maintain them at 27°C (eg by placing them on a warming tray).

12 Observe each Petri dish at t = 2 h after commencement of the test by examination under the microscope. Do not change positions of the dishes on the tray and return dishes immediately to the incubator after:

a) counting and recording the number of individual hydra (ie with or without buds);

b) noting if tentacles appear clubbed or contracted;

c) noting any other observations that suggest the hydra are not behaving or developing normally.

Observations are recorded at t = 2 h on the data sheets. To avoid observer bias, select a different replicate to observe each day. Also, commence observations with the next highest chemical concentration to that observed on the previous day (see below).

Note: that water movement will cause temporary tentacle contraction; allow the water to settle before recording observations.

Day 2

13 Dispense test solutions into appropriately labelled 100 mL vials and check the pH. If they are not within the prescribed limits, adjust accordingly using 0.05 M H_2SO_4 (1.39 mL per 500 mL) or 0.05 M NaOH (1 g per 500 mL).

14 When the pH range is established, dispense test solutions into appropriately labelled 45 mL vials (3 x 35 mL of each solution). Cap, and allow the dispensed solutions to equilibrate to 27°C.

15 Approximately twenty hours after the commencement of the test, remove the trays from the incubator, sort the test dishes into replicate groups (ie 3 groups), observe under the microscope and record as Day 1 observations.

16 After recording observations (as Day 1 observations) for that dish (as in step 14), feed each hydra in the dish.

Hydra are fed individually with at least 3–4 live brine shrimp nauplii (*Artemia franciscana*: see Appendix G). The nauplii are rinsed and suspended in synthetic water and placed in each dish using a glass Pasteur pipette. Feeding is allowed to proceed *ad libitum* for at least 30 minutes, but is generally best left for 2–3 h.

17 After all hydra have been observed and fed in the 18 dishes, place the test dishes onto trays in the random order for the day (see below), and return the trays to the appropriate position in the incubator.

18 Twenty-four hours after the commencement of the test, solutions are renewed as follows:

a) the test solution is swirled around the Petri dish to dislodge any uneaten brine shrimp and regurgitated food;

b) the solution is then tipped carefully into a second Petri dish (or cleaning dish);

c) an aliquot of the test solution (5 mL) is immediately added to cover the bottom of the test dish, the swirling process is repeated, and the solution tipped into the cleaning dish;

d) the remaining fresh solution (30 mL) is immediately added to the test dish;

e) any hydra that are dislodged into the cleaning dish are carefully picked up with a little water using a clean pipette and returned to the test dish;

f) any remaining brine shrimp, or other debris, in the test dish are removed by pipette, with care taken to minimise removal of test solution;

g) the cleaning dish is checked again for hydra, with any found being returned to the test dish; and

h) the solution in the cleaning dish is collected for the measurement of water parameters in each treatment after 24 h.

Note: Ensure that cross-contamination does not occur by obtaining a new pipette and cleaning dish whenever a dish of lower chemical concentration is cleaned after a higher concentration.

19 Measure the physical water parameters (ie pH, conductivity, dissolved oxygen) at the end of 24 h.

Day 3–4

20 Repeat steps 12–19 (ie measure and adjust synthetic water if necessary, count and record observations for the appropriate day, and feed at 24 h after step 14; clean and renew test solutions after step 18).

Measure the physical water parameters and record for the appropriate day.

On each day a new set of random numbers must be used for the position of each Petri dish in the incubator for the next 24 h period (see below).

Day 5

21 Count and record observations on each test dish 96 h (4 x 24 h) after the start of the test. Do not feed hydra and do not renew test solutions.

22 Measure the physical water parameters and record as Day 4.

Test is complete.

On each day a new set of random numbers must be used for the position of each Petri dish in the incubator for the next 24 h period (see below). Randomness is an important component of the experimental design. Random distribution of hydroids is achieved via steps 4–7. The Petri dishes are randomly assigned to positions on trays each day. Since the Petri dishes have a random position on the trays, they will also have a random position in the incubator. Random numbers are obtained from a random number table or generator for each day of the test; a set of random numbers is not to be reused. When the hydra have to be observed, then the Petri dishes can be sorted into replicate groups for greater convenience. This avoids the continual changing of glass pipettes by working through the water changes from a lower to a higher chemical concentration. At the end of the water changes the Petri dishes are then again randomly placed on trays and returned to the incubator.

To avoid observer bias there should be at least two observers. Each observer randomly selects a replicate group to record each day, and observations commence with the next highest chemical concentration to that which was first observed the previous day. Occasional checks should be made on the incubator performance (ie constant temperature and light intensity and their variation) by

placing replicates in different incubators. If appreciable differences are found, then the incubator that produces the most reliable and consistent results.

C.1.11 Acceptability of test data

The test data are considered acceptable if:

1 The recorded temperature of the incubator remains within the prescribed limits;

2 The mean mortality of the combined Control does not exceed 20%;

3 Greater than 80% of the surviving hydra in the combined Control are free swimming and healthy on completion of the test;

4 The recorded pH is within the prescribed limits;

5 The dissolved oxygen concentration was greater than 70% of the air saturation value throughout the test at 27°C;

6 The conductivity for each test solution was within ± 10% of the values obtained on Day 1; and

7 If the presence of fungus on hydra does not exceed 20% in any combined treatment group.

Statistical testing should not proceed if fewer than four groups (including Control) remain.

C.2 Purple-spotted gudgeon (*M. mogurnda*) sac-fry test

C.2.1 Objective

The objective of the test is to determine the concentration of a specified chemical that shows:

a) no effect (ie concentration showing no statistical difference ($P \leq 0.05$) between exposed and unexposed or control specimens) (measured using the 10% bounded effect concentration, BEC_{10}; see Hoekstra & Van Ewijk 1993); and

b) a lowest effect (ie concentration showing the smallest statistical difference ($P \leq 0.05$) between exposed and unexposed or control specimens) (measured as the minimum detectable effect concentration, MDEC; see Ahsanullah & Williams 1991) on the survival of newly hatched purple-spotted gudgeon sac-fry over 96 h.

C.2.2 Principle of the test

Recently hatched sac-fry (<10 h old) are exposed to a range of chemical concentrations for 96 h. Observations of any fry mortality are recorded at 24 h intervals. The method is based on the Gudgeon Embryo Larval Test described by Hyne et al (1996).

C.2.3 Test organism

The test species is *Mogurnda mogurnda* (Teleostomi, Eleotrididae) commonly known as the purple-spotted or northern trout gudgeon (Merrick & Schmida 1984). This carnivorous species is widely distributed throughout northern Australia (Merrick & Schmida 1984). The recommended husbandry method for *M. mogurnda* is described in Appendix E. Fertilised eggs are allowed to be guarded by the male parent in the aquarium for a 1–2 d. They are then removed and placed in a beaker (~2 L) containing half parent tank water and half synthetic test water, and allowed to hatch at 27 ± 1°C on a warming tray in the laboratory (see Appendix H). Gentle aeration (via an airstone) is used to simulate the male parent fanning water over the eggs to reduce the incidence of fungal spores settling. The eggs hatch after 3–4 d. Sac-fry (< 10 h old) are used as the test species and are obtained from laboratory

stocks. The embryos and sac-fry are *not* treated for fungus with malachite green but should be free from overt disease or gross morphological deformity. No feeding is required during the test. Animals obtain sufficient nutrition from the attached yolk-sac.

C.2.4 Synthetic water

See Section C.1.4.

C.2.5 Stock solutions

See Section C.1.5.

C.2.6 Test solutions

See Section C.1.6.

C.2.7 Apparatus and test equipment

See Section C.1.7.

C.2.8 Test environment

See Section C.1.8.

C.2.9 Data recording

See Section C.1.9.

C.2.10 Test procedure

Day 1

1 Prepare the test solutions (as outlined in Section 1.6) and leave at room temperature.

2 Isolate approximately 220 suitable test sac-fry in synthetic water in 3 Petri dishes and leave at room temperature. A 'suitable test sac-fry' is less than 10 h old at the commencement of the test ie no more than 10 h have elapsed since the time of hatching. The sac-fry may be seen as a developed, hatched fry lying on the bottom of the hatching container, with a prominent yolk-sac and black-eye pigmentation visible.

3 Dispense 30 mL aliquots of each test concentration (normally 7) into 3 appropriately labelled replicate Petri dishes (ie 3 x 30 mL for each test solution), and arrange in three replicate groups on clear plastic trays (eg Control Replicate 1 to X $\mu g\ L^{-1}$ on Tray 1).

4 Using a microscope and wide-mouth pipette, pick out one sac-fry from the isolated stock and place into Control Replicate 1.

5 Repeat for remaining test concentrations of replicate 1, working up in concentration, and ending with the highest concentration.

6 Discard the used pipette and select a new one.

7 Repeat steps 4–6 until all test dishes for that replicate group contain 10 sac-fry.

8 Observe each dish under the microscope to ensure that there are 10 sac-fry in each dish, and replace any sac-fry that are damaged in any way (eg disrupted yolk etc).

9 Repeat steps 4–8 for the remaining two replicate groups.

More than one person can distribute test sac-fry simultaneously, with the distribution appropriately split into replicate groups.

10 Cover the dishes and place them in the random order for that day (see below), in the positions 1 to 21.

11 Place trays in the incubator.

Completion of this stage constitutes the start of the test (time = 0 h).

Note: Whenever test dishes are removed from the incubator maintain them at 27°C (eg by placing them on a warming tray).

Day 2

12 Dispense test solutions into appropriately labelled 100 mL vials and check the pH. If they are not within the prescribed limits, adjust accordingly using 0.05 M H_2SO_4 (1.39 mL per 500 mL) or 0.05 M NaOH (1g per 500 mL).

13 When the pH range is established, dispense test solutions into appropriately labelled 45 L vials (3 x 30 mL of each solution). Cap, and allow the dispensed solutions to equilibrate to 27°C.

14 Twenty-four hours after the commencement of the test, remove the trays from the incubator, sort the test dishes into replicate groups (ie 3 groups), observe under the microscope and record the following as Day 1 observations:

a) count and record the number of live sac-fry;

b) count and record the number of dead and/or fungoid sac-fry; and

c) make any other observations that suggest that the sac-fry are not developing normally.

To avoid observer bias, a different set of replicates are to be observed first each day. Also, commence observations with the next highest concentration to that which was first observed the previous day (see below).

15 After observing a dish, the test solution is renewed as follows:

a) solution in the test dish is carefully emptied into a second Petri dish (or cleaning dish) with a gentle swirling action, tilting the dish to one side to pool the sac-fry in a small area;

b) enough of the appropriate fresh test solution (5 mL) is immediately added to cover the bottom of the test dish, the swirling process is repeated, and the solution pipetted or carefully tipped into the cleaning dish. Keep the sac-fry submerged at all times by tilting the dish;

c) the remaining fresh solution (30 mL) is then immediately added to the test dish;

d) any live sac-fry that are transferred to the cleaning dish at this stage are carefully put back into the test dish using a pipette;

e) any dead sac-fry in the test dish are removed with a pipette before renewal of test solution, with care taken to minimise removal of test solution. A fresh pipette is obtained after the removal of dead sac-fry;

f) the cleaning dish is checked again for sac-fry, with any found being returned to the test dish; and

g) the solution in the cleaning dish is collected for measurement of the physical water parameters in each treatment after 24 h (step 18).

Ensure that cross-contamination does not occur by obtaining a new pipette and cleaning dish whenever a dish of lower chemical concentration is cleaned after a high concentration.

16 After all dishes have been observed and test solutions renewed, place dishes in the random order for that day (see below), and return trays to the incubator.

17 Measure the physical water parameters at the end of 24 h (Day 1).

Day 3

18 Repeat steps 12–17 (ie at 24 h intervals count, record, renew test solutions and record the water parameters for the appropriate day) for 96 h to conclude the test.

On each day a new set of random numbers must be used for the position of each Petri dish in the incubator for the next 24 h period (see below). Randomness is an important component of the experimental design. Random distribution of sac-fry is achieved via steps 4–7. The Petri dishes are randomly assigned to positions on trays each day. Since the Petri dishes have a random position on the trays, they will also have a random position in the incubator. Random numbers are obtained from a random number table or generator for each day of the test; a set of random numbers is not to be reused. When the sac-fry have to be observed, then the Petri dishes can be sorted into replicate groups for greater convenience. This avoids the continual changing of glass pipettes by working through the water changes from a lower to a higher chemical concentration. At the end of the water changes the Petri dishes are then again randomly placed on trays and returned to the incubator.

To avoid observer bias there should be at least two observers. Each observer randomly selects a replicate group to record each day, and observations commence with the next highest chemical concentration to that which was first observed the previous day. Occasional checks should be made on the incubator performance (ie constant temperature, light intensity, and their variation) by placing replicates in different incubators. If appreciable differences are found, then the incubator that produces the most reliable and consistent results.

C.2.11 Acceptability of test data

See Section C.1.11.

Appendix D Raw data for final day toxicity test results

Table 1 Hydra—uranium

Conc (ug L^{-1})/ Test No.	0.1 Control	10	20	30	40	50	60	70	80	90	100	110	120	150	180	200	250	300	1000
280																			
rep1	30										24			19		7	2	0	
rep2	30										22			24		13	9	0	
rep3	35										28			22		16	2	0	
281																			
rep1	20	21		20							16							0	0
rep2	26	22		22							17							0	0
rep3	25	23		24							9							0	0
282																			
rep1	24	20		19							17							0	0
rep2	20	27		22							17							0	0
rep3	20	23		18							13							0	0
283																			
rep1	38			36		25		22		20		19		2					
rep2	35			37		22		22		18		16		0					
rep3	34			34		25		21		22		20		0					
283																			
rep1	31	35		23		19		25		14		0							
rep2	39	33		22		21		21		15		0							
rep3	35	31		22		21		15		3		0							

Table 1 cont

285									
rep1	34	33	35	28	25	27	18		
rep2	31	33	35	24	25	22	19		
rep3	30	31	35	24	23	22	21		
286									
rep1	28	28	30	23	20	23	25		
rep2	32	33	39	23	18	21	21		
rep3	29	33	36	30	23	24	22		
287									
rep1	35	35	18	0	0	0	0		
rep2	34	32	16	20	0	0	0		
rep3	34	37	20	2	0	0	0		
288									
rep1	37	35	22	18	0	0	0		
rep2	35	37	10	4	0	0	0		
rep3	36	28	20	5	0	0	0		

Table 2 Hydra—copper

Conc (ug L^{-1})/ Test no.	0.7 Control	1	1.5	2.5	3	3.5	4.5	5	6	8	10	15	20
300													
rep1	21							14			0	0	0
rep2	26							17			0	0	0
rep3	24							19			0	0	0
305													
rep1	36		18		20		0		0	0	0		
rep2	26		19		19		8		0	0	0		
rep3	29		19		19		2		0	0	0		
306													
rep1	29		21		21		4		1	0	0		
rep2	22		21		21		13		2	0	0		
rep3	27		20		19		13		0	0	0		
307													
rep1	28	20	21	21		19	13		2				
rep2	32	24	21	20		19	19		4				
rep3	29	22	22	20		19	19		2				
308													
rep1	26	19	20	22		20	17		0				
rep2	31	19	23	20		19	20		10				
rep3	27	20	22	22		18	18		1				

Table 3 Gudgeon—uranium

Conc (ugL^{-1})/ Test No.	0.1 Control	10	30	100	300	600	800	1000	1200	1350	1500	1650
295												
rep1	10	9	10	10	10			7				
rep2	10	10	10	10	10			9				
rep3	10	10	10	10	10			3				
296												
rep1	10	10	10	10	10			6				
rep2	10	9	10	10	10			8				
rep3	10	9	10	10	10			8				
298												
rep1	10				10	10	10	10	10		7	
rep2	10				9	10	10	10	8		5	
299												
rep1	10				10	10	10	10	10		5	
rep2	10				10	8	9	10	10		2	
rep3	10				10	8	10	9	10		4	
312												
rep1	10								10	10	7	9
rep2	9								10	9	10	9
rep3	10								10	10	9	10
313												
rep1	10								10	9	9	8
rep2	10								9	10	10	10
rep3	10								10	10	8	5
321												
rep1	2									7	0	0
rep2	10									7	0	2
rep3	10									7	2	1
rep4	10									5	0	0
rep5	10									10	2	0
rep6	10									7	2	1
323												
rep1	8							2	1	0	0	0
rep2	10							3	1	0	0	0
rep3	9							5	0	1	0	0
rep4	9							2	1	2	0	0
325												
rep1	10							10	9	5	7	0
rep2	10							10	5	8	2	1
rep3	10							10	7	4	8	1
rep4	10							10	7	7	5	0
rep5	10							10	9	8	6	1
rep6	10							10	7	7	9	1

Table 4 Gudgeon—copper

Conc (ug L^{-1})/ Test No.	0.7 Control	2	3	4	6	8	9	10	12	15	20	30
292												
rep1	10		10					5				0
rep2	10		10					5				0
rep3	10		10					4				0
293												
rep1	10		10					5				0
rep2	10		10					1				0
rep3	10		10					2				0
303												
rep1	10		6		1		2		1	2	0	
rep2	10		6		5		6		4	4	0	
rep3	9		7		1		4		1	3	3	
304												
rep1	10		10		5		4		2	5	0	
rep2	9		7		3		2		3	2	2	
rep3	10		6		2		3		1	3	0	
309												
rep1	10	10		8	9	10		10	9			
rep2	10	10		10	10	10		9	8			
rep3	10	10		10	10	8		10	8			
311												
rep1	10		10		9		10		7	8	7	
rep2	10		10		10		9		9	9	5	
rep3	10		8		9		9		6	8	6	
316												
rep1	10						9		8	7	2	1
rep2	10						8		8	6	1	0
rep3	10						9		7	8	1	1
rep4	10						9		7	8	4	2
rep5	10						10		8	9	3	1
rep6	10						10		7	8	3	0
322												
rep1	10						10		10	10	9	7
rep2	10						10		9	10	4	0
rep3	10						9		10	10	9	1
rep4	10						10		10	9	8	1
rep5	10						10		10	10	7	3
rep6	7						7		6	7	2	0

Appendix E Summary data for concentration-response relationships

Table 1 Uranium—*M. mogumda*

	U (µg L^{-1})[a]	% Survival	95% CI
1	117	100	0
2	356	100	0
3	709	100	0
4	938	100	0
5	1190	97	0
6	1264	92	2.7
7	1392	85	4.85
8	1557	60	9.7
9	1619	39	6.7
10	1724	19	7.3

[a] U expressed as uranyl (UO_2)

Table 2 Uranium—*H. viridissima*

	U (µg L^{-1})[a]	% Population growth	95% CI
1	1	100	0
2	22.8	100	2
3	34.2	100	2
4	45.6	98	2
5	57	93	5
6	68.4	80	8
7	79.8	74	13
8	91.2	69	13
9	102.6	60	11
10	114	48	10
11	125.4	31	8
12	136.8	19	5
13	171	4	1
14	342	0	0
15	1140	0	0

[a] U expressed as uranyl (UO_2)

Table 3 Copper—*M. mogumda*

	Cu (µg L^{-1})	% Survival	95% CI
1	0.7	100	0
2	2.2	100	0
3	3.13	99	2.1
4	4.23	98	2.3
5	6.11	97	1.7
6	8.22	96	1.7
7	10.22	95	2.1
8	12.3	91	2.3
9	15.44	86	2.4
10	20.55	60	3.2
11	25.22	34	2.4
12	30.67	17	3.1

Table 4 Copper—*H. viridissima*

	Cu (µg L^{-1})	% Population growth	95% CI
1	0.7	100	0
2	1	100	0
3	1.5	94	2
4	2.5	81	3
5	3	70	4
6	3.5	64	5
7	4.5	48	6
8	5	35	6
9	6	19	5
10	8	5	1
11	10	0	0
12	15	0	0

Appendix F Production of test hydra

Green hydra (*Hydra viridissima*) are cultured in the laboratory in bubble-aerated synthetic water (same source as test) within 'Gladwrap' covered, ventilated, 2 L glass bowls (primary stock). The culture water is taken from the same batch of synthetic water that is used to commence the test. The water movement caused by the gentle aeration results in most hydra attaching to the sides of the bowl via the basal disc, thus reducing time taken to perform water changes. Reserve (backup) stock hydra are maintained in tap water in back-up aquaria at a separate location, as a precaution against unknown chemicals or accidents occurring with the synthetic water. The backup aquaria are maintained as a 'community' tank, with 3 to 4 small fish (eg *Ambassis* sp., *Melanotaenia* sp.) and snails present.

Primary stock hydra are fed three times a week. One week prior to commencement of a test, they are fed daily to achieve maximum budding rates. Prior to commencement of feeding, hydra are observed and notes recorded in the primary hydra stock log book. A sample of water is then taken and the 'old' dissolved oxygen (DO) levels are recorded. Hydra are then fed with a thoroughly washed suspension of newly-hatched brine shrimp nauplii (*Artemia franciscana*—see Appendix G). The brine shrimp are re-suspended in synthetic water, and are pipetted into each primary stock bowl, a procedure designed to distribute them evenly over the hydra. The hydra are allowed to feed for at least 30 mins, and up to 4 to 5 h when possible. Six hours later, any uneaten brine shrimp and regurgitated food pellets are removed by swirling the water around each bowl and emptying it into a second cleaning dish (eg 4 L plastic container). More synthetic water is added and the procedure repeated until each bowl is free of brine shrimp. The bowls are then re-filled with clean water (approximately 1.5 L). Any hydra removed by the process are pipetted back into their glass bowl containing the fresh water. This process is referred to as a 'rinse' clean.

Stock bowls are cleaned at least twice weekly by performing a 'scrub' clean. After observations are made and recorded, and old DO samples are taken, excess water is carefully decanted away, ensuring that minimal hydra are lost. If necessary, the old water can be decanted into a cleaning dish. The bowls are then cleaned by gently pushing the attached hydra away from the sides of the bowl, and into a cleaning dish. Clean hands, or gloves can be used to carry out this procedure. The detached hydra are allowed to settle into a corner of the cleaning dish by slightly tipping it. Using a glass Pasteur pipette the hydra can then be transferred to a clean glass bowl containing fresh water. Backup hydra stock are fed daily with brine shrimp, and cleaned at least once a week. Excess hydra are gently pushed away from the sides of the aquaria and siphoned out, with a one-third water replacement. Bowls are washed by dishwasher (Gallay). Immediately prior to use, the bowls are rinsed with fresh synthetic water.

Periodically hydra are observed to reproduce sexually, making it difficult to maintain an isogenic population. The frequency with which this occurs can sometimes be reduced by introducing higher feeding rates and cleaning of the primary cultures, thus avoiding fouling of the water and fungal growth on the uneaten brine shrimp. If fungal contamination is observed at any time, the bowls can be given a rinse clean. Cladocera (*Moinodaphnia macleayi*) are fed at least once a week to the primary and backup hydra cultures as a natural diet supplement.

Appendix G Production of live brine shrimp larvae

Brine shrimp (*Artemia franciscana,* Utah—USA strain) are used as food for feeding many types of aquatic organisms, including larval fish and hydra. Brine shrimp can be cultured in a variety of containers to give an uninterrupted supply of nauplii (juvenile brine shrimp). The most appropriate type of culture containers are conical flasks (conical 1 L separation funnels are ideal) which, when inverted with the neck downwards, can be bubble-aerated from the bottom with oil-free compressed air. A 1 L salt solution is made by dissolving 30 g of coarse rock salt, or sea salt, in 1 L of warm water (30°C). After the salt is fully dissolved, one teaspoon (approximately 5 g) of commercially harvested dried brine shrimp cysts are added. Vigorous bubbling from the bottom of the container prevents eggs from settling.

Brine shrimp eggs will hatch in 18–24 h at an incubation temperature of 28°C when placed in an outside shaded position. At lower temperatures, hatching is delayed. On cloudy days the culture may need to be directly illuminated by a fluorescent lamp, since hatching is light dependent. To harvest the newly-hatched nauplii, the compressed air is turned off after 24 h (average water temperature of about 28°C) to allow the nauplii to settle and the empty egg shells to float. After 5 mins, the nauplii are strained through a fine nylon mesh net which is able to retain the nauplii, and they are washed with the test dilution water. The washed nauplii are then suspended in a small volume of dilution water (about 5 mL) and placed in a small beaker or Petri dish which is inclined at an angle of approximately 45° towards the light. Live nauplii will concentrate in the upper layer, while the unhatched cysts will remain on the bottom surface. The upper layer, containing live nauplii, is then collected for feeding. A Pasteur pipette or syringe is used to distribute the nauplii.

Appendix H Recommended husbandry method for *M. mogurnda*

Purple-spotted gudgeons (*Mogurnda mogurnda*) are collected from local waterways within the Magela Creek system of the Alligator Rivers Region, NT, Australia. Fish are captured either by baited fish traps or by fine meshed dip nets or seine nets, and are brought back to the aquaculture facilities at ***eriss***. Initially they are placed in either 80 L or 200 L aquaria; the number of fish in each aquarium is determined by the size of the fish. Observations are then made for a nominal period to ascertain fish health and acclimation to laboratory conditions, and also to determine the sex of the fish based on physical appearance of the papilla. Once the sex has been determined, the fish are divided into breeding groups, consisting of one male and either one, two or three females per aquarium. Further observations are then carried out to assess the breeding groups for fecundity, fertility and embryo hatchability to avoid any site-specific trait interfering with a test. Aquaria used for the fish are filled initially with tap water, and then 'modified' for the production of test embryos by the addition of either chilled deionised water, natural creek water, or synthetic water (ie chilled low conductivity water representing a storm event). The aquaria are located in a shaded aquaculture area outside the main testing laboratory; the water temperature in the aquaria during the Dry season ranges from 24 to 28°C, whereas during the build-up and subsequent wet season it ranges from 26 to 32°C. A cooler temperature is maintained during the warmer months by the addition of chilled water during a water change. Undergravel filters provide aeration coupled with a natural photoperiod.

Fish are fed once daily on a varied diet consisting of 'commercial fish pellet' (Aristo Pet high protein fish pellets) supplemented with live food when possible (eg tadpoles, water boatmen etc). It has been observed that such a diet is adequate to provide sufficient nutrition to the breeding fish and enable the continuous production of embryos for weeks at a time. In addition, it has been observed that the quality of the water in the aquaria can be maintained at a higher level with less fouling when using such food. Live food, such as tadpoles, can be captured and placed with the fish, allowing the fish to continue eating *ad libitum*. The aquaria are cleaned on a fortnightly basis (or more frequently as required) using a wide mouth vacuum siphon. The gravel is disturbed, allowing trapped leftover food, faeces and any other debris to be removed. To ensure fish are not subject to undue stress, a quarter or one-third water change is performed, and the water replaced either with chilled low conductivity water or tap water at ambient temperature.

The aquaria are set up in a row within the shaded aquaculture area, running along an east-west aspect. Washed gravel covers the bottom of the tanks, and a local green weed grows near the surface of the water providing refuge. Six washed black plastic plant pots with a diameter of 23 cm are placed in the tanks. Gravel or small stones are placed inside the pots to anchor them, and the opening of each pot is directed towards the front of the viewing area to assist observation. The pots provide a 'cave' refuge for the fish, and also a spawning surface. The male will select a spawning site (sometimes, however, it is the back of the thermometer, a rock, or the side of a tank), and the female lays a batch of eggs while the male fertilises them. Each day prior to feeding, the tanks are carefully observed for the presence of newly spawned eggs with the aid of a torch. The eggs are tubular in shape, have transparent cases, and are generally laid in circular patches of various sizes depending on the size of the breeding female. The egg batches range in size from 300–1000 eggs. The eggs are left in the aquarium to be guarded by the male parent fish for 24–48 h after being laid. They are then removed from the breeding aquarium and either kept and reared as future in-house bred

breeding stock, or are placed in a 2 L beaker containing half parent tank water and half test diluent water and allowed to hatch under laboratory conditions for use in a toxicity test (see Appendix I). To determine the age of the embryos they are observed under a stereo microscope while still covered with water. If the eggs are laid on a surface such as the wall of a pot, eggs can be removed for observation by carefully sliding a glass cover slip under the egg mass and moving it forward until the edge has some eggs attached to it. The cover slip with the eggs is transferred to a Petri dish with enough water to cover them while observations are made.

Gudgeon breeding is variable, however it is possible to gauge an approximate prediction on the production of a batch of eggs based on careful observation of both behaviour and physical characteristics of a group of fish (ie courtship behaviour accompanied by distinct golden colouration on the abdomen of the breeding female, and swelling and protrusion of both male and female papilla). It is advantageous to have at least 5 or 6 breeding aquaria set up and running so that sac-fry can be obtained for use in a toxicity test when needed. If a breeding group cease spawning, the fish can be swapped into different tanks with different combinations of groups of females. Alternatively, spawning can be delayed in a tank by placing a partition in it such that the male is isolated from the breeding females. After the partition is removed, it has been observed that spawning recommences within 1–2 d. This is beneficial for obtaining fish early in the week. If there is excessive disturbance or pedestrian traffic around the aquaria, opaque Perspex screens can be positioned around an area of an aquarium that is being used for spawning.

Appendix I Isolation of *M. mogurnda* sac-fry

When a batch of eggs is produced, the eggs are left in the parent tank for 24–48 h allowing the male parent fish to guard them. Infrequently the eggs may be eaten before they can be removed, however, it is noted that this is the exception rather than the rule, and may be due to the presence of excessive numbers of water mites (eg Suborder Oribatida) and microcrustacea in the breeding aquaria which invade and feed on the egg mass. To reduce the numbers of such fauna, a small black-striped rainbowfish (*Melanotaenia nigrans*) can be placed in a breeding aquaria.

After 24–48 h development in the parent tank, the developing embryos are carefully removed by placing the pot or rock etc, into a 2 L beaker containing half parent tank water and half diluent water, ensuring that the temperature of this water is ± 1°C of the parent tank water. The batch of developing embryos is then placed on a warming tray set at 27 ± 1°C in the laboratory to continue development. They are observed for deformities, viability or water mites etc. An airstone is positioned beneath the egg batch such that a gentle stream of bubbles passes upward over the surface of the eggs, simulating the fanning action of the male parent over the eggs to keep fungal spores from settling. The beaker is loosely covered with Gladwrap to stop dust etc. Frequent daily observations are made, ensuring minimal disturbance until hatching occurs. Half water changes are performed using test diluent water to ensure fouling does not occur. It takes approximately 10 h for the entire batch of eggs to hatch into sac-fry. After all the eggs have hatched (or at least sufficient numbers to enable a test to be commenced), they are carefully isolated into Petri dishes using a glass Pasteur pipette with an internal diameter at least 2 mm. Enough sac-fry are placed in each Petri dish so that there are enough for each replicate to be started. Any damaged sac-fry are discarded.