Comparison of the sensitivity of Australasian and non-Australasian aquatic organisms to selected metals

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

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ABSTRACT

The difference in sensitivity of Australasian species and their non-Australasian counterparts has not been thoroughly examined. Of those studies that have been undertaken, there was no clear pattern evident regarding which group of species was the most sensitive. The current study aimed to determine if there were any significant differences between the sensitivity of organisms from these two regions by collating metal toxicity data and determining if significant differences were evident using Student t-tests and species sensitivity distribution (SSD) methods. Generally, there was more non-Australasian toxicity data available than Australasian data. Therefore, the availability of sufficient toxicity data for Australasian species determined which metals could be investigated. The metals for which there was sufficient data were As(III), As(V), Cd, Cr(VI), Cu, Pb, Hg, U and Zn for freshwater organisms and Cd, Cr(VI), Cu, Pb, Hg, Ni, and Zn for marine/estuarine organisms. Data was assessed using quality assessment criteria that were tested and improved as part of this study. The quality of the toxicity data was assessed in order to ensure that only acceptable quality data were used in the comparisons. Statistical comparisons of the best available freshwater data revealed that 35% of the comparisons had significant differences ($p < 0.05$), with 80% of these, the Australasian species were the more sensitive. For the best available marine/estuarine water data, 47% of the comparisons showed significant differences ($p < 0.05$), with 60% of these, the non-Australasian organisms were more sensitive. Examination of the ratios of the differences between organisms from the two regions indicated that, as a whole, the freshwater Australasian species were significantly more sensitive while there were no significant differences ($p > 0.05$) detected between the marine/estuarine organisms.

SSDs could be derived for Cd, Cu and Zn in both fresh and marine/estuarine waters using acute toxicity data. Australasian freshwater organisms exposed to Cu were found to be significantly ($p<0.05$) more sensitive than the non-Australasian organisms. The five other comparisons showed no significant differences ($p > 0.05$). Estimated chronic trigger values (ECTVs) were derived using acute to chronic ratios. When comparing these ECTVs the Australasian organisms were found to be significantly more sensitive ($p < 0.05$) to Cu in freshwater, while the non-Australasian organisms were found to be significantly more sensitive ($p < 0.05$) to Cd in freshwater. The four other comparisons did not reveal any significant differences ($p > 0.05$).

Assessment factors were calculated using the ratio of the sensitivity of Australasian and non-Australasian species to the selected metals and then plotting the cumulative frequencies against the ratio. This analysis revealed that an assessment factor of 7.1 would need to be applied to protect 95% of Australasian organisms in freshwater ecosystems from 95% of chemicals studied, while an assessment factor of 2.2 would be needed to ensure that 95% of Australasian marine/estuarine organisms would be adequately protected from 95% of chemicals studied when using non-Australasian toxicity data to derive trigger values. The observed differences in sensitivity of Australasian and non-Australasian organisms to metals indicate that using non-Australasian data could cause either over or under protection of the local species and that this kind of study should be conducted with other chemical groups.

1 INTRODUCTION

The generation of Australasian aquatic toxicity data is of high importance. These data are used in hazard and risk assessments, either prospectively or retrospectively, to derive trigger values for water quality guidelines (WQGs), and ultimately to better manage and protect aquatic ecosystems. However, the paucity of Australasian aquatic toxicity data has meant that the above processes have to predominantly rely on non-Australasian (North American and European) data. By using non-Australasian data we are making an inherent assumption that the sensitivities of species from these regions are not different to Australasian species. Canada and the USA require that most toxicity data used to derive WQGs be from North America (CCREM 1999; USEPA 1994). From this, it could be inferred that they consider that aquatic organisms from North America may not be adequately protected by trigger values derived from non-North American toxicity data. A similar concern has been raised in a number of other regions including Australia (Johnston *et al.* 1990, Sunderam *et al.* 1992, Davies *et al.* 1994, Mulhall 1997, Markich and Camilleri 1997, Rose *et al.* 1998, Westbury *et al.* 2004, Phyu 2004) and Europe (Maltby *et al.* 2003), as well as tropical (Leung *et al.* 2003, Kwok et al. 2007) and polar regions (Chapman and Riddle 2003). The possible reasons why there may be differences in sensitivity of organisms from different regions include, climatic, biogeographical and physicochemical factors (see below) that can alter the uptake and toxicity of a chemical.

1.1 FACTORS THAT MAY CAUSE DIFFERENCES IN TOXICITY DATA BETWEEN AUSTRALASIAN AND NON-AUSTRALASIAN SPECIES

1.1.1 Climate

There are substantial temperature differentials over the Earth, a large proportion of which are due to variation in the incoming solar radiation. These temperature differences coupled with precipitation per annum are considered two of the most important environmental variables pertaining to regional climate. The five major climate types, as outlined by the Koppen climate classification system are: tropical, dry, temperate, continental, and polar climates (http://en.wikipedia.org/wiki/Koppen_climate_classification). The local climate has a

large bearing on the distribution of species and has a large effect on abiotic factors that may enhance or decrease the toxicity of a chemical in a water body. The Australasian region, which is comprised of Australia (excluding its Antarctic territories), New Zealand, Papua New Guinea, Malaysia, Indonesia, the Philippines and smaller islands in the South Pacific Ocean (Markich *et al.* 2002), shares all of the Koppen climate types with the non-Australasian region, with the exception of the polar climate. But there is very limited toxicity data for polar species (Chapman and Riddle, 2003) and none of it has been used to derive WQGs. Therefore it would appear unlikely that climate could, at least at the scale being considered in this study, cause differences in sensitivity between Australasian and non-Australasian species.

1.1.2 Biogeography

The geographic distribution of species, or biogeography, may influence the relative sensitivity of organisms to a chemical. It is well known that continents and countries have an array of organisms that are endemic, but it is also not uncommon to find the same, or very similar, organisms or taxa in different regions (e.g. *Ceriodaphnia dubia* and *Pseudokirchneriella subcapita* (formerly *Selenastrum caprocornutum*) which are both cosmopolitan species). Given that there may be a great range of organisms used for toxicity testing from one taxon or phylum (e.g. Crustacea, Chordata), it would be expected that differences in sensitivity from one region to another may be observed. This could mean that an important factor causing a difference in sensitivities of organisms to chemicals from Australasian and non-Australasian regions is due to the inherently different species.

Given the geologically long period that Australia has been isolated from most other land masses, it is quite possible that Australia may contain species that are only distantly related to those from North America and Europe. The fact that Australia contains many unique animals supports this hypothesis. Calabrese and Baldwin (1993) examined the size of the assessment factor that would need to be applied to protect 95% and 99% of species (i.e. the interspecies assessment factor) in terms of how closely related the organisms were that would be protected. They found that as the species became less closely related, the size of the interspecies assessment factor increased (Table 1.1).

Similar findings have also been made by Le Blanc (1984) and Barnthouse *et al.* (1990). While these findings do not prove or disprove that there are inherent differences in the sensitivity of the Australasian and non-Australasian species to metals, it does suggest that distantly related species may have different sensitivities to different substances.

Table 1.1 The interspecies assessment factors (AF) for a range of different interspecies extrapolations that should protect 95 and 99% of the species. Taken from Warne (1998)

Type of interspecies extrapolation	Interspecies assessment factors to protect a theoretical percentage of all species	
	95%	99%
Species within Genus	10.0	16.3
Genera within Family	11.7	16.9
Families within Order	99.5	145.0
Orders within Class	64.8	87.5
Classes within Phyla	1000[1]	

[1] Obtained from Slooff *et al.* (1986); all the other interspecies AF values were obtained from Calabrese and Baldwin (1993).

1.1.3 Physicochemical factors

Physicochemical characteristics of water, such as temperature, pH, hardness, alkalinity, conductivity, dissolved oxygen (DO) and dissolved organic matter (DOM), may influence the toxicity of a chemical (e.g. Johnston *et al.* 1990, Erickson *et al.* 1996, Markich *et al.* 2001). The effect that each of these parameters has on the toxicity of metals is described below.

There seems to be no single pattern for effects of temperature on toxicity of chemicals to aquatic organisms. Temperature change in a given direction may increase, decrease or cause no change in toxicity, depending on the toxicant, the species, and in many cases the experimenter who has selected the particular procedure or response (Sprague 1985). For example, Del Ramo *et al.* (1987) found that the toxicity of cadmium (Cd) and mercury (Hg) to the freshwater crayfish *Procambarus clarkii* increased with increasing temperature. Conversely, Hansen *et al.* (2002) found that the toxicity of copper (Cu) to bull trout (*Salvelinus confluentus*) and rainbow trout (*Oncorhynchus mykiss*) decreased when the temperature increased (i.e. from 8°C to 16°C).

pH is an important parameter that may influence metal bioavailability in freshwater. A decrease in the pH may have both a direct and an indirect effect on metal toxicity. A direct effect of pH is the liberation of free carbon dioxide, particularly from hard waters, which can quickly become toxic to aquatic organisms. An indirect effect is a scenario where a decrease in pH can increase the free metal ion concentration, resulting in metal desorption from colloidal and particulate matter, and dissociation of some inorganic and organic metal complexes (Markich *et al.* 2001). It is widely considered that an increase in proton (H^+) concentration competitively inhibits the binding of the free metal ion (e.g. Cd^{2+}) at the cell membrane surface (Campbell 1995) and hence, reduces metal bioavailability.

Water hardness is the measure of dissolved cations, predominantly calcium and to a lesser extent magnesium, in freshwater. The bioavailability of cadmium (Cd), Cu, chromium III (Cr (III)), nickel (Ni), lead (Pb) and zinc (Zn) in freshwater typically decreases with increasing water hardness (Markich *et al.* 2001), with calcium (Ca) and/or magnesium (Mg) competing with metals for surface binding sites on cell membranes (Markich and Jeffree 1994) and also by reducing the concentration of the free metal ion in solution (Campbell 1995). However, Markich *et al.* (2005) have found that a freshwater alga (*Chlorella* sp.), a bacterium (*Erwinnia* sp.) and a cladoceran (*Ceriodaphnia* cf. *dubia*) exposed to Cu in synthetic and natural freshwaters of varying hardness (43 to 375 mg $CaCO_3$/L), with constant alkalinity, pH and dissolved organic carbon concentration, demonstrated an absence of hardness effects in the pH range 6.1 to 7.8. This result coupled with findings from other investigations (Riethmuller *et al.* 2000, Charles *et al.* 2002, Markich *et al.* 2005) indicates an absence of significant hardness effect when selected freshwater biota are exposed to Cu at environmentally-relevant concentrations.

Alkalinity is a measure of the buffering capacity of water, or the capacity of bases to neutralise acids. Alkalinity does not refer to pH, but instead refers to the ability of water to resist a change in pH. The presence of buffering materials helps neutralisation of acids as they are added to the water. These buffering materials are primarily the bases bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and occasionally hydroxide (OH^-), borates, silicates, phosphates, ammonium, sulfides, and organic ligands. Alkalinity directly affects metal speciation in solution through complexation with carbonate, which affects

metal bioavailability (Erickson *et al.* 1996) and hence should affect metal toxicity. In their physiological investigations of Cu toxicity, Lauren and McDonald (1986) concluded that alkalinity, but not pH, affected short-term lethality of Cu to the Rainbow trout.

Conductivity is a measure of the inorganic materials and inorganic ions in water; of which total soluble salts form a major component, and thus conductivity is used to measure salinity of water. Chloride, phosphate, sulfate, and nitrate anions (negative ions) as well as calcium, magnesium, iron, aluminum, and sodium cations (positive ions) contribute to overall conductivity and can decrease the toxicity of some chemicals including metals through both buffering and competition (Markich *et al.* 2001).

The DO concentration measured in a water-body reflects the equilibrium between processes that consume oxygen (e.g. respiration) and those that release oxygen (e.g. photosynthesis and the physical transfer of oxygen from the atmosphere to the water-body). Measurements of DO may indicate a disturbance to these competing processes and define the living conditions for organisms that require oxygen to live (ANZECC & ARMCANZ 2000). At reduced DO concentrations, many toxic compounds in water become more toxic; the acute toxicity of several common toxicants roughly doubles as the DO concentration was halved from 10 mg/L to 5 mg/L (EIFAC 1973). The toxicities of Zn, Pb, Cu, ammonia, cyanide, hydrogen sulfide and pentachlorophenol all increased at low DO concentrations (Davis 1975).

Humic substances are a major component of natural dissolved organic matter (DOM) in aquatic systems (Vaughan *et al.* 1993). They comprise a physically and chemically heterogenous mixture of relatively high molecular weight organic compounds, formed by secondary synthesis reactions (humification) during the decay process and transformation of biomolecules that originate from dead organisms and microbial activity (Senesi 1993). The toxicity of metals decreases when in the presence of DOM as some of the free metal ion will complex with these materials and become unavailable to cause toxic effects (Kim *et al.* 2001).

From the preceding, it is clear that a range of physicochemical factors can modify the toxicity of metals. But in order for physicochemical factors to cause or contribute to

differences in the sensitivity of Australasian and Non-Australasian species, there must be differences in the values of the physicochemical parameters between these two regions. The vital question is therefore: are there differences in the physicochemical properties of Australasian and Non-Australasian marine and freshwaters. Johnston *et al.* (1990) described freshwater Australasian conditions as markedly different from the world average or world standard freshwaters. Examples of these differences include: in general the water temperatures in Australia are higher than in Europe or North America: low flow rates of Australian rivers contribute to a higher concentration of natural organic matter: sodium and chloride ions dominate Australian freshwaters which is in contrast to elsewhere which are dominated by divalent cations and bicarbonate; Australian waters have lower amounts of DO due to the warmer water temperature and hydrogen ion concentration (pH) can vary greatly from around 5 in Magela Creek, Northern Territory to 9 in the Murray River. All of these factors can either reduce or enhance the toxicity of a chemical depending on the characteristics of the chemical.

The marine environment as a whole does not vary in any great degree in its physicochemical composition due to the buffering effect of the carbon dioxide - carbonic acid – bicarbonate system acting as buffer to keep the pH of seawater within a narrow range (Nybakken 1988). Other factors that may have an effect are temperature and salinity which have been mentioned above and localised factors such as the input or lack of input of water from rivers and streams and other sources.

Therefore the likelihood of these factors modifying toxicity between Australasian and non-Australasian species is open to conjecture but it is certainly possible.

1.1.4 Methods to reduce the influence of factors on toxicity

Standardisation has been widely incorporated into toxicity testing to minimise variability that may be caused by the aforementioned factors, leaving the sensitivity of the organism as the only source of variability. The use of water fleas (cladocerans) as a test species is widely accepted in many countries around the world as a 'standard' test organism. In the USA, *Daphnia pulex* is the water flea most commonly employed in these tests, while in Europe *D. magna* is more commonly used (Mark and Solbe 1998).

In Australia *Ceriodaphnia* cf. *dubia* is the most commonly used water flea (Chapman *et al.* 2001). There are a number of regulatory bodies (mainly located in the northern hemisphere) that have published toxicity test protocols [e.g. Environment Canada (EC 1996), the Organisation for Economic Co-operation and Development (OECD 1998), and the American Society for Testing and Materials (ASTM 2001)].

The initial step to standardisation is a thorough understanding of the various chemical, physical and biological factors that affect toxicity results. Standardisation can then be achieved by the adoption of detailed test protocols that minimise or standardise disturbing factors, use of a standard test species, selection of specific test types designed to meet specific objectives and the use of reference toxicants or certified test animals. A standardised method with reference toxicants and standard test species should theoretically maximise comparability, replicability and reliability and is essential for answering questions of relative toxicity and sensitivity or replicability of tests (Buikema *et al.* 1982).

1.2 COMPARISONS OF THE SENSITIVITY OF GROUPS OF ORGANISMS

As mentioned earlier, other countries have been concerned about using toxicity data for species not present in their ecosystems. There has also been concern about organisms from different ecosystems (e.g. static and flowing waterbodies) and different media (e.g. salt and fresh water) can be used to protect different systems. However, very few studies have actually attempted to resolve this issue. In the following two examples of this type of research conducted outside Australasia are presented.

A study was conducted by Maltby *et al.* (2003), as one of its objectives, to determine whether there were differences in the cumulative species sensitivity distributions of organisms from different ecosystems, continents and media. The authors found that there were enough data to compare the response of freshwater and saltwater arthropods for six insecticides, flowing water and standing water arthropods for four insecticides, tropical and temperate arthropods for three insecticides, and European and North American arthropods for three insecticides. It was concluded that there was no significant ($p > 0.05$) difference in the cumulative distribution function for arthropods

from temperate and tropical regions, from Europe or North America, or from flowing water or standing water habitats. Therefore, the authors tentatively concluded that it would be relatively safe to use North American ecotoxicity data to derive water quality guidelines for European ecosystems, and vice versa, for a selected range of insecticides.

Leung *et al.* (2003) compared the sensitivity of tropical and temperate freshwater species to nine metals, seven pesticides, phenol and ammonia using species sensitivity distributions (SSD). The author found that the metals silver, cadmium, chromium, copper, mercury, nickel and lead were more toxic to temperate than tropical species, while zinc and arsenic were more toxic to tropical than temperate species. The pesticides, carbaryl, chlorpyrifos, DDT, lindane and malathion were more toxic to temperate than tropical species while chlordane and pentachlorophenol phenol and ammonia were more toxic to tropical than temperate species. Leung *et al.* (2003) concluded that temperate species could be used to derive predicted no effect concentrations (PNECs) for tropical species in most cases, but for chemicals that have never been tested on tropical species, a safety factor of 20 - 40 should be applied with a view to protecting tropical freshwater ecosystems.

1.3 Comparisons of the sensitivity of Australasian and non-Australasian species to toxicants

There have been few studies on differences in chemical sensitivities between Australasian and non-Australasian species. A summary of the findings of these studies is presented in Table 1.2. Johnston *et al.* (1990) investigated the applicability of the Organisation of Economic Co-operation and Development (OECD) test procedures for deriving Australian water quality criteria for phenol, pentachlorophenol and 1,1,2-trichlorethane. Representative species from three freshwater trophic levels (one alga, eight cladocerans and four fish) were tested in Sydney tap water. The authors tested six Australasian and two non-Australasian cladocerans and two Australasian and two non-Australasian fish and an alga. By comparing whether or not confidence intervals overlapped they concluded that in no case were the Australasian species more sensitive than the non-Australasian species at temperatures up to 25°C (the maximum temperature tested).

Sunderam *et al.* (1992) found that the non-Australasian European carp (*Cyprinus carpio*), was more sensitive to endosulfan than the native eastern rainbowfish, (*Melanoteania duboulayi*) and silver perch (*Bidyanus bidyanus*). They concluded that 'in the case of endosulfan and Australian fish, it is not strictly necessary to determine toxicity to native species in order to derive water-quality criteria'.

Davies *et al.* (1994) exposed non-Australasian rainbow trout (*Oncorhynchus mykiss*) and two native fish, the common jollytail (*Galaxias maculatus*) and the blenny (*Pseudaphritis urvillii*), to low concentrations of seven pesticides under identical conditions. The pesticides tested were the organophosphate insecticides, acephate and fenitrothion; the phenoxyacetate herbicide MCPA-Na; the triazine herbicides, atrazine and cyanazine; the fungicide chlorothalonil, and the pyrethroid insecticide, cypermethrin. They found from interspecies comparisons (using the Wilcoxon signed-rank test) that water quality criteria based on juvenile *O. mykiss* toxicity data alone would not be suitable for the protection of juvenile and adult *G. maculatus* and *P. urvillii* from physiological stress in at least four of the seven cases.

Mulhall (1997) compared the sensitivities of the Australasian freshwater cladoceran, *C.* cf. *dubia* and the non-Australasian cladoceran, *D. magna* to 14 polar narcotics, comprising phenol and benzamine derivatives. While for some chemicals the *C.* cf. *dubia* was more sensitive, for others it was not. On average however, *D. magna* was 1.5 times more sensitive that the Australasian *C.* cf. *dubia*; thus the relative sensitivity of *C.* cf. *dubia* and *D. magna* to the chemicals was similar.

Markich and Camilleri (1997) found that there was no significant difference in the acute toxicity of copper and uranium to the Australian tropical freshwater fish, the purple spotted gudgeon (*Mogurnda mogurnda*) and the temperate freshwater fish, the common jollytail (*Galaxius maculatus*). The authors concluded that the Australian water quality guidelines for copper, derived largely from North American toxicity data, were appropriate for Australian conditions when key water quality variables (eg. temperature, water hardness and alkalinity) are considered.

In contrast to this finding, Rose *et al.* (1998) found that *C.* cf. *dubia*, was approximately three times more sensitive than *D. magna* to a range of 17 non-polar narcotic chemicals (benzene, 1,2-dichlorobenzene, 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene, pentachlorobenzene, 4-chlorotoluene, 2,4-dichlorotoluene, *m*-xylene, 1,2-dichloropropane, 1,2,3-trichloropropane, 2-ethoxyethanol, monochlorobenzene, 1,4-dichlorobenzene, pentanol, octanol, nonanol, heptanol) when tested under the same conditions.

Westbury *et al.* (2004) examined the toxicity of twelve substituted phenols (different to those studied by Mulhall) that had a polar narcotic mode of action to the Australasian cladoceran *C.* cf. *dubia* and the American *D. magna* plus seven other species. They found that by regressing the toxicity of the two species, *D. magna* was on average 1.2 times more sensitive that *C.* cf. *dubia*; again a similar chemical sensitivity.

Phyu (2004) applied the Burr Type III species sensitivity method (Campbell *et al.* 2000) to data on the sensitivity of Australasian and non-Australasian aquatic species to the herbicides, atrazine and molinate. In that study, trigger values were calculated in accordance with the methods used to derive the Australian and New Zealand water quality guidelines for toxicants (ANZECC and ARMCANZ 2000). Trigger values were calculated in a number of different ways and in each case they indicated that there were no significant ($p > 0.05$) differences in the sensitivity between Australasian and non-Australasian species to the two test chemicals.

Hose and Van den Brink (2004) compared the sensitivity of fish, invertebrates and amphibians to the organochlorine insecticide, endosulfan, using SSDs, and also investigated whether SSDs derived from laboratory data would adequately protect organisms in the field. They found that there was no significant difference between the Australasian and non-Australasian fish and arthropods, and that laboratory data adequately protected organisms in the field.

Table 1.2 Summary of the results of studies, on the relative sensitivities of Australasian and Non-Australasian species.

Study	Chemicals	Sensitivity
Johnston *et al.* (1990)	Organics	Aust ≤ Non-Aust
Sunderam *et al.* (1992)	Pesticides	Aust < Non-Aust
Davies *et al.* (1994)	Pesticides	Aust > Non-Aust
Mulhall (1997)	Polar narcotics	Aust < Non-Aust
Markich and Camilleri (1997)	Cu and U	Aust = Non-Aust
Rose *et al.* (1998)	Non-polar narcotics	Aust > Non-Aust
Westbury *et al.* (2004)	Polar narcotics	Aust < Non-Aust
Phyu (2004)	Atrazine and Molinate	Aust = Non-Aust
Hose and Van den Brink (2004)	Endosulfan	Aust = Non-Aust

Collectively these studies indicate no overall pattern to the relative sensitivity of Australasian and non-Australasian organisms to a variety of chemicals. In addition, these studies have a number of limitations. Firstly, some contain data for many species but few chemicals (i.e. Johnston *et al.* 1990, Sunderam 1992, Phyu 2004, Hose and Van den Brink 2004). Secondly, some have data for a reasonable number of chemicals but few species (i.e. Rose *et al.* 1998, Davies *et al.* 1994, Mulhall 1997). Thirdly, some lack rigorous statistical analyses (i.e. Sunderam *et al.* 1992, Mulhall 1997, Rose *et al.* 1998, Westbury *et al.* 2004). Due to these limitations, it is currently not possible to determine with any degree of confidence, whether there are differences in the sensitivity of Australasian and non-Australasian aquatic species to toxicants.

In order to conduct a definitive study to resolve whether there are differences in the sensitivity of Australasian and non-Australasian species, the above limitations must be overcome. What is required is sufficient toxicity data for both Australasian and non-Australasian species for a range of chemicals and rigorous statistical techniques to be applied.

Examination of the Australasian Ecotoxicity Database (AED) indicated that the group of chemicals for which there is the most comprehensive data is metals and metalloids. There is also relatively a large amount of metal toxicity data for a wide variety of non-Australasian species. It was therefore decided to examine metal toxicity data in a definitive study to determine if there are differences in the sensitivity between Australasian and non-Australasian species.

1.4 METALS IN AQUATIC ENVIRONMENTS

Metals are ubiquitous in the environment. Many are essential for mediating biological functions in organisms. Essential trace metals, such as Co, Cu, Fe, Mn, Ni, or Zn, are involved in many of the enzymatic and metabolic reactions that take place within an organism, either as a component or an activator of enzymes (Lehninger 1982). However, some metals, such as Hg, Pb, and U, have no known biological function, and therefore are classified as non-essential. All metals, whether essential or non-essential to an organism, become toxic beyond certain threshold concentrations (Depledge *et al.* 1994).

The dawn of the Industrial Revolution brought about an unprecedented demand for metallic items for use in human health and welfare, the industrial economy and protection of national security (Nriagu 1994). While the benefits of metals and their compounds are indisputable, the ecological price of their use is only beginning to be acknowledged. Each year, large quantities of metal wastes are discharged into the environment. The number of metallic compounds, as well as the quantity of the industrial discharges, continues to grow (Thornton 1996). In fact, it has been estimated that the environmental toxicity due to metallic wastes (mobilised by human activities) exceeds the combined toxicity of all radioactive and organic wastes (Nriagu and Pacyna 1988).

Major sources of anthropogenic metallic inputs into aquatic environments include domestic and industrial waste-waters, sewage discharges, urban runoff and atmospheric fallout (Nriagu 1990). The sustained input of metals into aquatic environments has resulted in a range of environmental problems on local, regional and global scales. On the basis of the relative sizes of the various environmental compartments, it can be inferred that freshwater environments are particularly at risk in terms of metal pollution (Nriagu 1990, United Nations 1997).

Assuming 10-20% of the global discharge into aquatic environments is into lakes and rivers (the remaining 80-90% of the loadings go directly into seas and coastal marine waters), the calculated pollution load would result in the dwarfing of natural

background (i.e. minimal anthropogenic disturbance) concentrations of trace metals in such ecosystems (Nriagu and Pacyna 1988). As trace metal concentrations in aquatic environments approach toxic thresholds for many organisms, the potential of wide-scale anthropogenic disturbance becomes evident.

1.5 OBJECTIVES

The overall objective of this study was to determine if there are differences in the sensitivity between Australasian and non-Australasian aquatic species to metals and metalloids.

The differences in sensitivity of the Australasian and non-Australasian species were investigated at different levels, these being:

- overall comparison of Australasian and non-Australasian biota;
- comparison of Australasian and non-Australasian biota by taxon;
- comparison of similar Australasian and non-Australasian species; and
- hardness modified data was also used in some of the above comparisons.

These comparisons were carried out using different statistical methods in order to gain as much information on the sensitivities of the two groups of organisms before a conclusion was made. These methods include Student's t-test and species sensitivity distributions.

2 GENERAL METHODS

2.1 INTRODUCTION

This project used two sets of data, the Australasian Ecotoxicity Database (AED) (Warne *et al.* 1998; Warne and Westbury 1999; Markich *et al.* 2002) and a non-Australasian Ecotoxicity Database (NAED), which was compiled as part of this work and used to make comparisons with the AED. The two datasets were used in a number of different ways to make comparisons of the sensitivity of Australasian and non-Australasian species to metals.

Australasia, as defined in this study, consists of two biogeographical regions – the Australian region (Australia and New Zealand) and the Oriental region (Papua New guinea, East Malaysia, Indonesia, the Philippines, and smaller islands in the South Pacific Ocean) (Simpson 1977; Dudgeon 1995). Animal and plant species of the Australian region are usually distinct from those of the Oriental region. For example, species of freshwater fish from Indonesia, Malaysia and the Philippines are more closely related to those from (tropical) mainland Asia (e.g. Thailand, West Malaysia, Burma, India) than Australia. Irian Jaya (now part of Indonesia) and Papua New Guinea represent a melting pot for Australian and Oriental species. However, the vast majority of biota inhabiting these islands are considered to be of Oriental derivation (Gressit 1982). The arbitrarily defined boundary between the Australian and Oriental regions is not based exclusively on the biogeographical distribution of particular species (Simpson 1977). Indeed, some species have a natural distribution that includes both geographical regions (e.g. Tiger prawn, *Penaeus monodon*).

Non-Australasia is, for the purposes of this study, defined as any country not belonging to Australasia. Most of the non-Australasian research papers from which metal toxicity data were extracted, originated from North America and Europe, with a small percentage from India.

2.2 DATABASE COMPILATION

The NAED was created using five steps:

1. selection of metals for comparisons;
2. collection of appropriate toxicity data;
3. screening the toxicity data;
4. assessing the quality of the toxicity data; and
5. inclusion of data into the database.

How each of these steps was conducted is explained in the following sections.

2.2.1 Selection of metals for comparisons

Toxicity data contained in the AED have been published for pesticides (Warne *et al.* 1998), organic chemicals (Warne and Westbury 1999) and metals (Markich *et al.* 2002) for Australasian species or non-native species tested under Australasian conditions. The metals AED was used to select the metals and taxonomic groups of organisms for which toxicity data were to be included in the NAED. For each metal and taxon combination (e.g. copper and Crustacea) to be included in the NAED, there needed to be a minimum of three data points available in the AED. The metals for which there were sufficient freshwater Australasian data were As(III), As(V), Cd, Cr(VI), Cu, Pb, Hg, U and Zn. The metals for which there were sufficient marine/estuarine Australasian data were Cd, Cr(VI), Cu, Pb, Hg, Ni, and Zn.

2.2.2 Collection of non-Australasian data

Non-Australasian toxicity data for those combinations of metal and taxon that met the above minimum data requirements were obtained by conducting searches of databases and the literature. The databases that were searched included the USEPA ECOTOX database (USEPA 2004) and the ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) database (ECETOC 1993). The literature searched included the NSW EPA in-house toxicity library, other water quality documents from Canada, the UK and the Netherlands and a broad range of abstracting services (e.g. AQUALINE,

Aquatic Sciences and Fisheries Abstracts, BIOSIS, Chemical Abstracts, Current Contents, INIS, Scifinder, Science Citation Index, Zoological Record). The search was limited to papers from 1970 to the end of 2002 due to advances in the filed of ecotoxicology in the last three decades.

2.2.3 Screening the toxicity data

The following data were excluded from the NAED:

- mixtures of metals;
- organometallics (e.g. tributyl tin, methyl mercury, methyl arsenate);
- metals in waste waters (e.g. mine or sewage effluents);
- bioaccumulation and residue studies;
- data that did not quantify toxic effects;
- unpublished work;
- the duration of the exposure was not stated;
- the toxicological endpoint was not stated; and
- the measure of toxicity was other than 50% effects.

2.2.4 Data Quality Assessment

The quality of each toxicity datum that did not meet the exclusion criteria was determined using a scoring method (Table 2.1) described by Markich *et al.* (2002), that was an expansion and improvement of the method used in the USEPA (2004) ECOTOX database. A very similar data assessment scheme/system was used to assess the quality of data used to derive the Australian and New Zealand water quality guidelines for toxicants (ANZECC and ARMCANZ 2000) and the organic chemical, pesticide, metal and metalloid components of the AED (Warne *et al.* 1998; Warne and Westbury 1999; Markich *et al.* 2002 respectively).

The quality of each datum point entered into the NAED was assessed using this scoring method. Each question in Table 2.1 was awarded one of the possible marks indicated in the second column. Half marks were not awarded in an attempt to reduce the subjectiveness of the assessment scheme. The marks awarded for each question were

summed to obtain a total score. Different aspects are important to the quality of different types of toxicity data for different types of chemicals (i.e. metals and non-metals). Thus, the total possible score for different combinations of chemicals and toxicity data are different (Table 2.1). The quality score (Table 2.1) for each datum was obtained by expressing the total score obtained as a percentage of the total possible score for that particular combination of data type and chemical (Table 2.1).

Table 2.1 The questions used and the marks awarded to assess the quality of toxicity data for aquatic biota. Taken from Markich *et al.* (2002).

Question		Mark
1	Was the duration of the exposure stated (e.g. 48 or 96 hours)?	10 or 0
2	Was the biological endpoint (e.g. immobilisation or population growth) defined?	10 or 0
3	Was the biological effect stated (e.g. LC[1] or NOEC[2])?	5 or 0
4	Was the biological effect quantified (e.g. 50% effect, 25% effect)? The effect for NOEC and LOEC[3] data must be quantified.	5 or 0
5	Were appropriate controls (e.g. a no-toxicant control and/or solvent control) used?	5 or 0
6	Was each control and chemical concentration at least duplicated?	5 or 0
7	Were test acceptability criteria stated (e.g. mortality in controls must not exceed a certain percentage) Invalid data must not be included in the database.	5 or 0
8	Were the characteristics of the test organism (e.g. length, mass, age) stated?	5 or 0
9	Was the type of test media used stated?	5 or 0
10	Was the type of exposure (e.g. static, flow through) stated?	4 or 0
11	Were the chemical concentrations measured?	4 or 0
12	Were parallel reference toxicant toxicity tests conducted?	4 or 0
13	Was there a concentration-response relationship either observable or stated?	4 or 0
14	Was an appropriate statistical method or model used to determine the toxicity?	4 or 0
15	For NOEC/LOEC/MDEC[3]/MATC[4] data was the significance level 0.05 or less? **OR** For LC/EC[5]/BEC[6] data was an estimate of variability provided?	4 or 0 4 or 0
16	For metals tested in freshwater (FW), was the pH, hardness, alkalinity and organic carbon content measured during the test and stated (3 marks each)? Award 1 mark if it is measured but not stated, or if the dilution water only is measured and stated. **OR** For all other chemicals, was the pH measured and stated (3 marks)? Award 1 mark if it is measured but not stated or if the dilution water only is measured and stated.	3, 1 or 0
17	For marine and estuarine water (MEW), was the salinity/conductivity measured and stated?	3 or 0
18	For tests not using aquatic macrophytes and alga, was the dissolved oxygen content of the test water measured during the test?	3 or 0
19	Was the temperature measured and stated?	3 or 0
20	Was the grade or purity of the test chemical stated?	3 or 0
	Total score	
	Total possible score for the various types of data and chemicals: FW/metal/non-plant = 100. FW/non-metal/non-plant = 91. FW/metal/plant = 97. FW/non-metal/plant = 88. MEW/non-plant = 91. MEW/plant = 88)	
	Quality score ([Total score ÷ Total possible score] 100)	
	Quality class (High ≥80%, 51-79% Acceptable, Unacceptable ≤ 50%)	

[1] Lethal Concentration; [2] No Observed Effect Concentration; [3] Lowest Observed Effect Concentration; [4] Minimum Detectable Effect Concentration; [5] Maximum Acceptable Toxicant Concentration; [6] Effective Concentration; [7] Biological Effect Concentration

It should be noted that if a paper contained different types of toxicity data (e.g. LC/EC and NOEC/LOEC) or if several different experimental designs were used, then a separate quality score was calculated for each type of data and experiment. Therefore, it is possible for there to be a number of quality scores for data from a single paper. The toxicity data were classified into three classes depending on the quality score. Data with a quality score of 50% or less, 51 to 79%, and 80% or greater were classed as unacceptable (U), acceptable (A) and high (H) quality, respectively.

The quality of the data was assessed in order to provide the readers with a quantitative measure of whether the data had been generated using appropriately rigorous scientific methods. Any comparison to be made between Australasian and non-Australasian ecotoxicity data requires the use of good quality data, otherwise incorrect conclusions may be drawn. For this reason, only 'acceptable' and 'high' quality data were used in this study for the comparisons of the sensitivity of Australasian and non-Australasian species.

2.2.5 Inclusion of data in the database

Those data that passed the exclusion criteria and were of acceptable or high quality were included in the NAED. The data in the NAED for freshwater and marine/estuarine species are presented in Appendices 1 and 2, respectively.

The following information was included in the NAED. The names of the metals were reported as the metal (e.g. Cu and Cr), rather than the metal salt (e.g. $CuSO_4$, $Cr_2O_7^{2-}$). The valency state of the metal was also reported, where relevant, as this is known to influence their toxicity to biota (e.g. Vaughan and Greenslade 1998). The common and scientific names of the test species and their taxa (division/phylum), the life stages or characteristics of the test species, (e.g. length and age), mode of exposure (i.e. static, static-renewal, flow-through), the exposure medium (e.g. synthetic or natural water) and its physicochemical properties (e.g. temperature, pH, salinity/conductivity, organic matter content), the duration of the exposure, the toxic effects (e.g. survival and population growth) and test endpoints (e.g. NOEC and EC50) measured, the concentration at which the toxic effects occurred, whether the concentration was

measured or nominal, the quality score (%) and references for the data were all recorded. A measure of the uncertainty of toxicity (i.e. 95% confidence limits, standard deviation or coefficient of variation) was also included when available.

2.3 TOXICITY DATA MANIPULATIONS

It is well documented that certain physicochemical factors play an important role in the bioavailability of certain metals in fresh surface waters (e.g. Stumm and Morgan 1996; Markich *et al.* 2001; Chapter 1 of this Thesis). For freshwaters these factors include water hardness, alkalinity, pH, DOM and redox potential (Markich *et al.* 2001). For marine/estuarine waters, DOM, redox potential and salinity/conductivity are important variables (e.g. Stumm and Morgan 1996). If toxicity data from the AED and NAED affected by such parameters are compared, then any differences in sensitivity that occur could be attributed to both inherent differences in sensitivity of the species as well as differences caused by these parameters. However, if the toxicity data are all standardised for each parameter before comparison, then any differences that occur should be mainly due to inherent differences in the sensitivity of Australasian and the non-Australasian species.

Water hardness is the only one of the above variables for which quantitative relationships with metal toxicity have been developed (Markich *et al.* 2001). Such relationships exist for Cd, Cu, Pb, Zn and are presented in Table 2.2. Therefore, by modifying the water hardness to a standard value, a comparison can be made between Australasian and non-Australasian species with confidence that any differences that are detected are mainly due to the inherent sensitivities of the organisms. A water hardness of 30 mg/L $CaCO_3$ was used for this purpose in the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ 2000) and was used for the present study.

Table 2.2 The hardness-dependent algorithms for freshwater (≤2.5‰) that were used in the ANZECC and ARMCANZ (2000) water quality guidelines and in this study.

Metal	Hardness-dependent algorithm[a]
Cadmium	$HMT = T(H/30)^{0.89}$
Copper	$HMT = T(H/30)^{0.85}$
Lead	$HMT = T(H/30)^{1.27}$
Zinc	$HMT = T(H/30)^{0.85}$

[a] HMT, hardness-modified toxicity (μg/L) at a hardness of 30 mg/L as $CaCO_3$; T, toxicity measured in the experiment; H, hardness of the water used in the experiment; Hardness is measured as mg/L as $CaCO_3$) in fresh surface water. Table has been modified from Markich *et al.* (2001).

2.4 STATISTICAL METHODS

The statistical methods that were used to determine if there were differences in the sensitivity of Australasian and non-Australasian aquatic species to metals and metalloids varied from chapter to chapter. Therefore details of these methods will be provided in the method sections of the experimental chapters.

3 EVALUATION OF CRITERIA USED TO ASSESS THE QUALITY OF AQUATIC TOXICITY DATA*

3.1 INTRODUCTION

Aquatic toxicity data form an important component of hazard and risk assessments and in the derivation of water quality guidelines (WQG), all of which are ultimately used to manage and protect aquatic ecosystems. However, the quality and reliability of the above processes depends to a large degree on the quality of the toxicity data used. Therefore, schemes that can assess the quality of toxicity data such as that used in the (USEPA ECOTOX database (USEPA 2004) and the Australasian Ecotoxicity database (AED) (Warne *et al.* 1998, Warne and Westbury 1999, Markich *et al.* 2002) were used to develop the current Australian and New Zealand WQG (ANZECC and ARMCANZ 2000). In these data assessment schemes, the quality of data presented in published or unpublished research papers is assessed by awarding scores based on a series of criteria or questions, designed to ascertain the scientific rigor of the testing provided in the paper. Table 2.1 provides the data assessment schemes used for the AED (Warne *et al.* 1998, Warne and Westbury 1999, Markich *et al.* 2002).

The quality and reliability of hazard and risk assessments and WQG can be improved by determining data that are of unacceptable quality and excluding their use. However, no matter how carefully a data quality assessment scheme is designed, there always remains the potential for different users to assign the same data different quality scores and/or quality class. This can occur in a number of ways: (a) assessors may have different interpretations of the information provided in the paper; (b) assessors may fail to find information provided in the paper; and (c) assessors may have different interpretations of the question and scoring scheme. Therefore, the aim of this study was to investigate whether variation in the quality assessment of toxicity data occurs between different assessors and, if so, to quantify this variation, analyse sources of this variation and if necessary, to modify the AED data quality assessment scheme. This was done by asking volunteer scientists working in the field of ecotoxicology to assess the

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* Published as: Hobbs DA, Warne MStJ, Markich SJ. 2005. Evaluation of criteria used to assess the quality of aquatic toxicity data. Integrated Environmental Assessment and Management 1:174-180

quality of data in two different research papers describing the toxicity of metals to aquatic organisms.

Although the weighted scoring used in the data assessment scheme presented in this study may be subjective and arguable, it was nonetheless used as a benchmark to evaluate the aforementioned aims of this study, and thus, was not the focus of the work. The approach (Markich *et al.* 2002) was modified from the scoring scheme used to evaluate the USEPA ECOTOX database (USEPA 2004), the only other ecotoxicity data quality assessment scheme known to the authors.

3.2 METHODS

The survey consisted of assessing the quality of data in two peer reviewed journal papers that reported the toxic effects of metals to aquatic organisms using the AED scoring scheme (Table 1). The two journal articles used were Buhl (1997) and Cheung and Lam (1998). Both papers were chosen randomly for the purpose of this study. The papers had been assessed as part of another project and were classed as having acceptable quality (i.e. a score of 51-79%; Hobbs *et al.* 2004). The survey was sent to 55 scientists, all of whom have some experience in the field of ecotoxicology. The selected participants included post-graduate students and scientists with varying degrees of ecotoxicological experience (i.e. 2-25 years) from government and non-government organizations.

In addition, Hobbs *et al.* (2005) of this study collectively scored each paper to establish a benchmark against which to evaluate the scores of the 52 other assessors participating in the survey. The answers to each question from the collective assessment of the three authors became the 'agreed responses', while the sum of the numerical responses became the 'agreed quality score' (AQS). The agreed responses and AQS were used as the answers and quality scores to which all other responses were compared in all subsequent analyses. An attempt was made to remove all subjectivity from the 'agreed responses' and AQS by critically assessing each paper jointly with the answer to each question proven to the satisfaction of all three authors. The authors of Hobbs *et al.* (2005) have a collective experience of assessing about 2,000 research articles using the

present scoring scheme.

The quality score and classification for each paper was determined by each of the 55 assessors using the scheme provided in Table 2.1. The variability of the quality scores for each paper was presented by plotting the quality scores against the respondent number and comparing scores to the AQS. Simple linear regression analysis was used to determine whether a relationship existed between the years of ecotoxicological experience of the assessors and the absolute value of the deviation from the AQS for each paper. The number of assessors that gave an answer different to the agreed response was determined for each question (Table 2.1). The responses and question were then examined to determine why different answers were given. The questions in the data assessment scheme were then modified in order to improve the usability of the scheme and to reduce assessor variation. A follow up survey using the revised assessment scheme was conducted using the same two papers (Buhl 1997; Cheung and Lam 1998) six months after the original survey. The survey was distributed to 13 of the original 55 assessors, and responses were received from 7 of those 13. It was not thought that the reduced number of assessors for the second survey would have a huge effect on the outcome of the comparisons of the two surveys and for the purposes of this study such error was ignored. Each assessor was asked to review each independently and to not refer to the original surveys.

3.3 RESULTS AND DISCUSSION

Twenty three surveys out of a possible 55 were returned (i.e. 42% return rate). The 23 surveys included the individual responses of the three authors (i.e. 20 other assessors). The AQS was 65 for Buhl (1997) and 70 for Cheung and Lam (1998). For the Buhl (1997) study, the median quality score awarded by the assessors was 70 and the mean and 95% confidence interval was 70 ± 3.1. The range of scores was 57 to 83. Figure 3.1 shows that 16 assessors gave the paper a higher mark than the AQS. Six assessors scored the paper lower than the AQS and one assessors gave the same score. For the Cheung and Lam (1998) study, the median quality score awarded by the assessors was 71 and the mean and 95% confidence interval was 69 ± 3.5. The scores ranged between 54 and 82. Figure 3.2 shows that 12 assessors scored the paper higher than the AQS, 10 awarded a lower score and one respondent gave the AQS score.

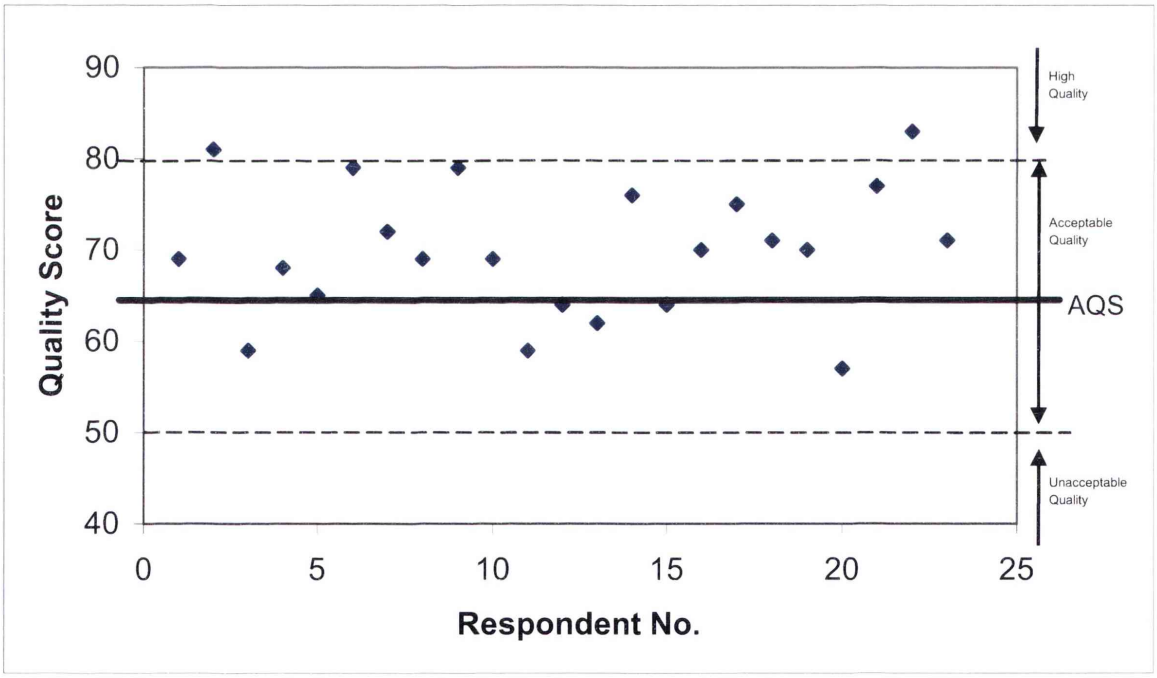

Figure 3.1 Quality score for each respondent assessing the Buhl (1997) study. The AQS is shown as the solid horizontal line and the quality classes indicated by the broken lines.

Despite the variation in scores awarded (Figure 3.1 and Figure 3.2), only two assessors for each paper (i.e. 8.7%) gave a score that would result in a different data quality classification. In four instances, the quality of the data was overestimated with the classification given as "High Quality" (i.e. > 80%). This low level of misclassification

of data quality suggests an overall robustness of the data quality assessment scheme and the ability of the assessors to form a common basis for judging the data.

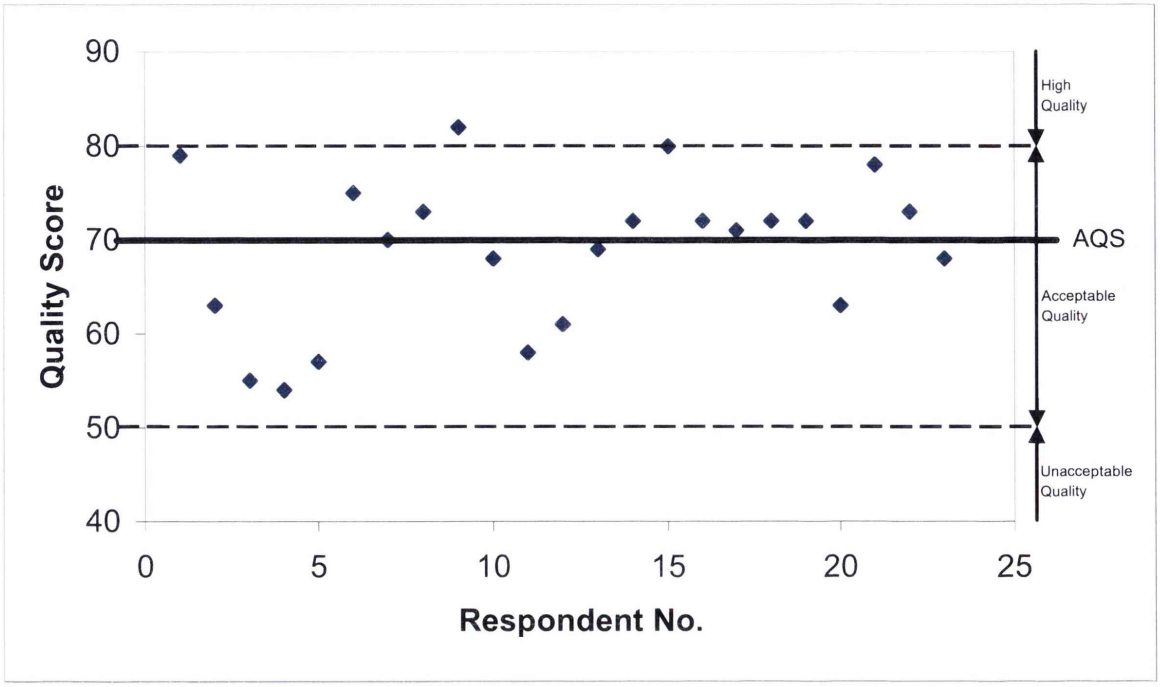

Figure 3.2 Quality score for each respondent assessing the Cheung and Lam (1998) study. The AQS is shown as the solid horizontal line and the quality classes indicated by the broken lines.

The relationship between the number of years of experience in ecotoxicology and the absolute deviation of the quality score from the AQS (Figure 3.3) was not significant ($p > 0.05$) for either paper (i.e. $p = 0.76$ for Buhl (1997) and $p= 0.051$ for Cheung and Lam (1998)). There was no significant ($p = 0.21$) relationship when AQS values for each paper were combined. Therefore, the degree of experience of the assessors did not affect the ability to assess the quality of the data.

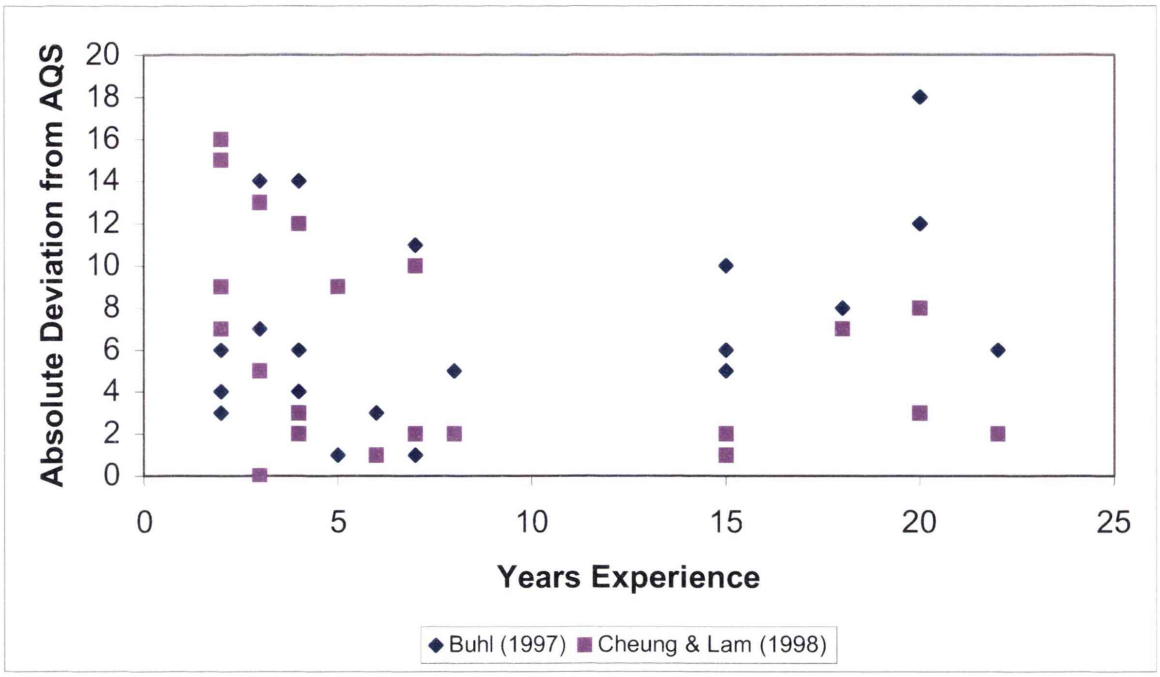

Figure 3.3 Absolute deviation of individual quality scores from the agreed quality score (AQS) for both studies as a function of the number of years of experience in ecotoxicology of the assessors.

The percentage of assessors that gave answers that differed from the agreed response for each question was examined, and is presented in Table 3.1. The authors in this study arbitrarily determined that those questions where more than 20% of assessors gave the "incorrect" answers (i.e. different from the agreed response) would be investigated further to determine the cause of the error. It was possible the question was ambiguous or poorly written or, alternatively, the assessors overlooked the information. It can be seen that questions 2, 7, 13 and 16 all had > 20% of assessors giving "incorrect" answers for Buhl (1997). For the Cheung and Lam (1998) paper, questions 7, 10, 11, 16 and 19 had > 20% of assessors giving "incorrect" answers.

Table 3.1 Percentage (%) of the 55 ecotoxicology assessors who answered questions that differed from the agreed response established by the authors to judge the data quality of two randomly selected ecotoxicity studies used in this paper to evaluate the data quality evaluation process.

Question No.	Buhl (1997)	Cheung and Lam (1998)
1	0	13
2	78*	0
3	0	0
4	13	4.3
5	4.3	0
6	13	0
7	26*	78*
8	0	4.3
9	0	4.3
10	4.3	43*
11	8.7	48*
12	0	0
13	30*	4.3
14	4.3	4.3
15	4.3	8.7
16	87*	100*
17	0	0
18	4.3	4.3
19	8.7	61*
20	8.7	4.3

* the authors believe that deviation greater than 20% from the agreed response for a question signifies a lack of consensus among assessors.

In some cases, it appears that the wording of the question led to the difference in responses. For example, question 2 asks if the biological endpoint is defined. Almost 80% of assessors answered that the biological endpoint had been defined in the Buhl (1997) paper, even though the endpoint was simply stated as "mortality" and a definition of mortality was not supplied. Thus, the agreed response for this question for Buhl (1997) was a value of zero. Nearly 61% of assessors responded to question 19 for the Cheung and Lam (1998) paper indicating that temperature had been measured in the testing and indicated in the protocol. However, Cheung and Lam (1998) stated that the environmental chamber was maintained at 25°C, but did not record the temperature either within the chamber or in any of the test solutions.

Other answers from the assessors that differed from the agreed responses may be due to the assessor not reading the paper carefully and, therefore, missing the relevant information or data. This was evident in both papers for question 16 and in Cheung and Lam (1998) for questions 10 and 11. Of all the questions in the assessment scheme, question 16 had the largest amount of variation in the answers provided by the assessors. This may be, in part, due to the question having multiple parts and multiple scores depending on the answers. Both papers provided at least some of the appropriate information. The Buhl (1997) paper clearly provides the physicochemical parameters of the test solution in Table 2, and the Cheung and Lam (1998) paper indicated the physicochemical parameters for the dilution water, but not for the test solutions.

For question 10 approximately 44% of the assessors missed that the acute tests were conducted under static conditions in the Cheung and Lam (1998) paper. Further confusion may have arisen when answering question 11 (i.e. whether the chemical concentrations had been measured) for the Cheung and Lam (1998) paper. The paper states that concentrations were measured 'at the time of solution renewal', which only occurred in the chronic tests. The concentrations were not measured for the acute tests.

For other questions, the cause for differences among survey assessors from the agreed response could only be attributed to assessor error. Question 7 asks whether test acceptability criteria were stated. For the Buhl (1997) paper, 26% of assessors declared that the test acceptability had been stated even though this was not the case. Assessors may have assumed that the first sentence of the results section stating 'there were no mortalities in any of the control treatments' was a test acceptability criteria. Technically, this is not a test acceptability criterion and, therefore, a score of zero was the appropriate response to question 7. For the Cheung and Lam (1998) paper, 78% of assessors stated incorrectly that test acceptability criteria had been provided. Similarly, for question 13, 30% of assessors stated that the Buhl (1997) paper had a concentration–response relationship either observable or stated, but neither was evident in the paper.

After examining the feedback from the assessors, it was clear that the AED quality assessment scheme was a useful and robust method for assessing the quality of data in ecotoxicology studies included in any database or used for any risk or hazard assessment. It was also clear that, in some cases, the assessment questions were

contributing to some degree to the variation in data quality scores. As a result questions 2, 7, 15, 16, 19 and 20 were revised to improve the clarity of the question (Table 2.1). For example, question 2 was reworded to include the option of awarding 5 marks if the biological endpoint was stated, but not defined (Table 3.2). This was done to help reduce the error of awarding marks in studies in which the biological endpoint is stated but not defined, as well as not penalising a paper for only stating the endpoint. A second option was included for question 7 to allow for 2 marks to be awarded to a study that did not state the test acceptability criteria in the results, but inferred that test acceptability criteria have been considered due to the test method used (e.g. USEPA, OECD and ASTM test methods contain test acceptability criteria).

Question 16 was rewritten as a 2-part question so that each physicochemical parameter for which data should be specified by a study are clearly indicated. These changes should make it less confusing for the assessors Table 3.2. Question 19 was modified to remove any confusion that may have arisen regarding whether the question referred to measuring the temperature of the test media or the test chamber (Table 3.2). Question 20 was reworded to encourage full marks for experiments that used chemicals of the highest available purity, irrespective of the actual level of purity (Table 3.2). To address the issue of assessors overlooking information that was stated, but not necessarily in the Methods section, a note was added to the data assessment scheme informing users to read the entire paper (Table 3.2).

The results of the follow-up survey that used the revised data assessment scheme showed that the mean variation of scores (and their range) from the AQS was reduced from 6.2 (original) to 4.2 (revised) (i.e. by 32%) for Buhl (1997) (Figure 3.4), but showed no improvement (i.e. 4.3 to 4.3; 0%) for Cheung and Lam (1998) (Figure 3.5). Perhaps, the lower original mean variation of scores for the Cheung and Lam (1998) paper reflect a high level of data quality and presentation of test information, which made it more difficult to improve upon the original data quality score.

Table 3.2 Revised scheme for assessing the quality of aquatic toxicity data with the modified questions underlined.

Note: To determine the quality of data, the entire article should be read

Question		Mark
1	Was the duration of the exposure stated (e.g. 48 or 96 hours)?	10 or 0
2	Was the biological endpoint (e.g. immobilisation or population growth) stated and defined (10 marks)? Award 5 marks if the biological endpoint is only stated	10, 5 or 0
3	Was the biological effect stated (e.g. LC[1] or NOEC[2])?	5 or 0
4	Was the biological effect quantified (e.g. 50% effect, 25% effect)? The effect for NOEC and LOEC[3] data must be quantified.	5 or 0
5	Were appropriate controls (e.g. a no-toxicant control and/or solvent control) used?	5 or 0
6	Was each control and chemical concentration at least duplicated?	5 or 0
7	Were test acceptability criteria stated (e.g. mortality in controls must not exceed a certain percentage) **OR** were test acceptability criteria inferred (e.g. test method used (USEPA, OECD, ASTM etc) uses validation criteria) (award 2 marks). Note: Invalid data must not be included in the database.	5, 2 or 0
8	Were the characteristics of the test organism (e.g. length, mass, age) stated?	5 or 0
9	Was the type of test media used stated?	5 or 0
10	Was the type of exposure (e.g. static, flow through) stated?	4 or 0
11	Were the chemical concentrations measured?	4 or 0
12	Were parallel reference toxicant toxicity tests conducted?	4 or 0
13	Was there a concentration-response relationship either observable or stated?	4 or 0
14	Was an appropriate statistical method or model used to determine the toxicity?	4 or 0
15a	For NOEC/LOEC/MDEC[4]/MATC[5] data was the significance level 0.05 or less? **OR**	4 or 0
15b	For LC/EC[6]/BEC[7] data was an estimate of variability provided?	4 or 0
16a	For metals tested in freshwater (FW), were the following parameters measured?	
(i)	pH,	3, 1 or 0
(ii)	hardness,	3, 1 or 0
(iii)	alkalinity and	3, 1 or 0
(iv)	organic carbon concentration Award 3 marks for each variable that was measured during the test and values stated. Award 1 mark for each parameter if it is measured but not stated or if they are measured and values are stated for the dilution water only. **OR**	3, 1 or 0
16b	For all other chemicals, was the pH measured and values stated? Award 1 mark if it is measured but not stated or if the pH of the dilution water only is measured and stated.	3, 1 or 0
17	For marine and estuarine water (MEW), was the salinity/conductivity measured and stated?	3 or 0
18	For tests not using aquatic macrophytes and alga, was the dissolved oxygen content of the test water measured during the test?	3 or 0
19	Was the temperature measured and stated (3 marks)? Award 1 mark if only the temperature settings of the room or chamber are stated.	3, 1 or 0
20	Were analytical reagent grade chemicals or the highest possible purity chemicals used for the experiment?	3 or 0
	Total score	
	Total possible score for the various types of data and chemicals: FW/metal/non-plant = 100. FW/non-metal/non-plant = 91. FW/metal/plant = 97. FW/non-metal/plant = 88. MEW/non-plant = 91. MEW/plant = 88)	
	Quality score (|Total score ÷ Total possible score|100)	
	Quality class (H ≥80%, 51-79% A, U ≤ 50%)	

[1] Lethal Concentration; [2] No Observed Effect Concentration; [3] Lowest Observed Effect Concentration; [4] Mnimum Detectable Effect Concentration; [5] Maximum Acceptable Toxicant Concentration; [6] Effective Concentration; [7] Biological Effect Concentration

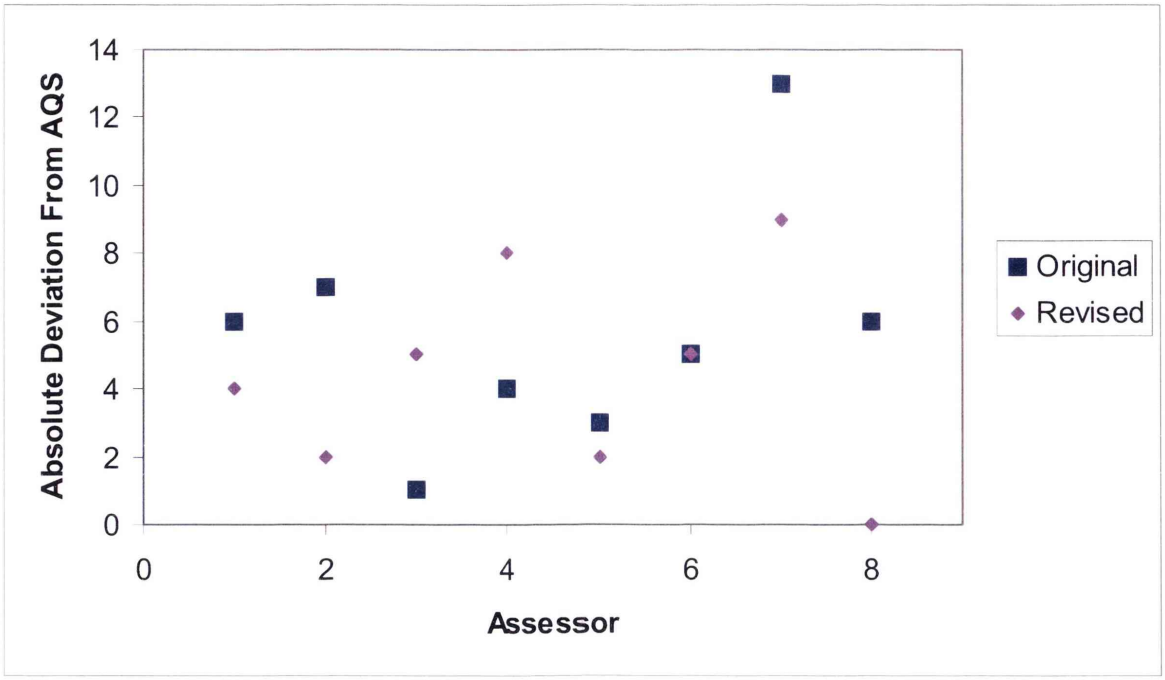

Figure 3.4 Absolute deviation of individual quality scores from the original and revised agreed quality scores (AQS) for the Buhl (1997) study.

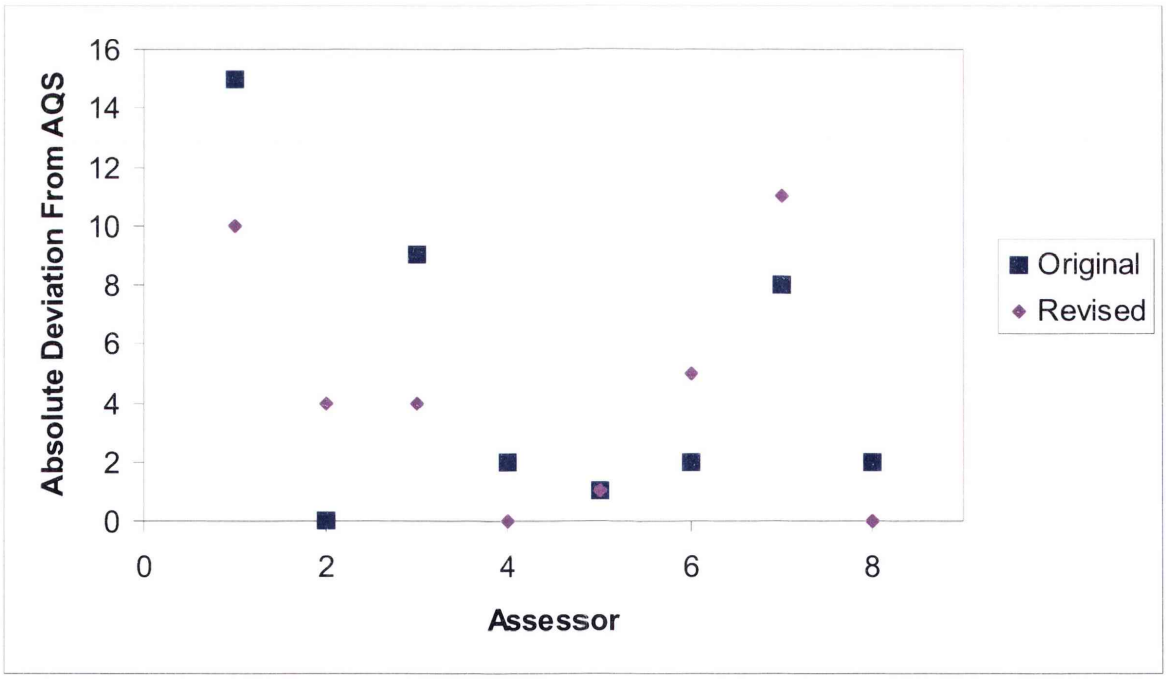

Figure 3.5 Absolute deviation of individual quality scores from the original and revised agreed quality scores (AQS) for the Cheung and Lam (1998) study.

3.4 CONCLUSIONS

- The AED quality assessment scheme should be an effective tool for assessors to confidently, no matter what their degree of ecotoxicological experience, assess and classify the quality of aquatic toxicity data reported in a published or unpublished research paper.
- In this study, the majority of the variation in the two assessment case studies occurred in responses to only five of the 20 questions used to evaluate data quality. In these five questions, the criteria appear to have been ambiguous or poorly written, the information difficult to find, the information overlooked by the assessor, or assessors interpreted the criteria differently.
- Revision of these questions in the AED quality assessment scheme led to improvement in the consensus of the assessors.

4 COMPARISON OF THE SENSITIVITY OF AUSTRALASIAN AND NON-AUSTRALASIAN SPECIES TO METALS USING 95% CONFIDENCE INTERVALS AND THE STUDENT'S T-TEST

4.1 INTRODUCTION

The generation of Australasian aquatic toxicity data is very important as they are used in hazard and risk assessments, either prospectively or retrospectively, to derive trigger values for water quality guidelines (WQG), and ultimately to manage and protect aquatic ecosystems. However, a paucity of Australasian aquatic toxicity data has meant that the above processes have had to predominantly rely on non-Australasian (i.e. North American and European) toxicity data. By using non-Australasian data in hazard and risk assessments of Australasian ecosystems, the inherent assumption has been made that the sensitivities of Australasian and non-Australasian species to toxicants are not significantly different. Canada and the USA require that most toxicity data used to derive WQG be from North America (CCREM 1999, USEPA 1994). From this, it could be inferred that they consider that aquatic organisms from North America may not be adequately protected by trigger values derived from non-North American toxicity data. A similar concern has been raised in a number of other regions including Australia (Johnston *et al.* 1990, Sunderam *et al.* 1992, Davies *et al.* 1994, Mulhall 1997, Markich and Camilleri 1997, Rose *et al.* 1998, Westbury *et al.* 2004, Phyu 2004) between Europe and North America (Maltby *et al.* 2003), between tropical and temperate regions (Leung *et al.* 2003, Kwok *et al.* 2007) and between polar and temperate regions (Chapman *et al.* 2006). There are a number of different approaches that could be used to statistically analyse the toxicity data from different regions and determine if there are differences in sensitivity. In this chapter, potential differences in the relative sensitivities of Australasian and non-Australasian organisms to a range of different metals, in both freshwater and marine/estuarine water, will be investigated using 95% confidence intervals and Student's t-test.

4.2 METHODS

Comparisons were made between the Australasian and non-Australasian groups of taxon and species. As there were few ubiquitous species (*Pseudokirchneirella subcapitat*) that could be compared in this project, species that are used in standardised toxicity testing from different regions (i.e. *Ceriodaphnia* cf. *dubia*, *Daphnia magna*, *Daphnia pulex*) or were similar types of organisms (*Cherax destructor*, *Cambarus robustus*) were used as well. For each comparison, the mean and 95% confidence interval for the mean were calculated for both Australasian and non-Australasian data. These values were used to determine if the groups of Australasian and non-Australasian data were significantly different or not. All calculations were carried out using the statistical package SPSS (2004). Error bar plots that summarised the above data, such as Figure 4.1, were generated for each comparison. If the 95% confidence limits of the two groups being compared did not overlap then they were considered significantly different (Barr, 1969; Lo, 1994; Nelson, 1989) and no further statistical analysis was required. Using non-overlapping 95% confidence intervals is a conservative method, and the probability of making a type I error (i.e. stating there is a difference when there in fact is no difference) is considerably less than 0.05. The actual probability does vary with the degrees of freedom, but with the degrees of freedom typically used in the comparisons in this chapter, the probability of a type I error occurring is approximately 0.01 (Ray Correll, *pers. comm.*). However, when 95% confidence limits overlap, then the two groups of data may or may not be significantly different (Barr, 1969; Lo, 1994; Nelson, 1989). Independent Student t-tests were used to determine whether there was a significant difference ($p \leq 0.05$) in sensitivities between Australasian and non-Australasian taxa. The Student t-test has some inherent assumptions that need to be considered, these being; the dependent variable is normally distributed, the two groups have approximately equal variance on the dependent variable and the two groups are independent of each other (Zar 1998). These assumptions were tested for each Students t-test with normality checked using a Kolmogorov-Smirnov test and a probability-probability plot (P-P plot) while the homogeneity of variance was checked using the F-ratio test. If the homogeneity of variance assumption was not met then a modified Students t-test was used that did not assume equal variance. If the normality assumptions were not met the non-parametric comparison Mann-Whitney test was used to detect for significant differences. A power analysis was undertaken for each

comparison using the statistical package NCSS (2004). This program uses the mean, standard deviation and number of samples to determine the power of the analysis to detect a significant difference between the two groups.

Unless otherwise stated, the level of significance for all statistical tests conducted in this chapter was $p = 0.05$. Therefore, unless specifically stated otherwise, all statements that a result is significantly different means the probability associated with this is ≤ 0.05 and not significantly different means the probability associated with this is > 0.05. A comparison of the sensitivity of Australasian and non-Australasian organisms was only made if both groups contained at least three data points.

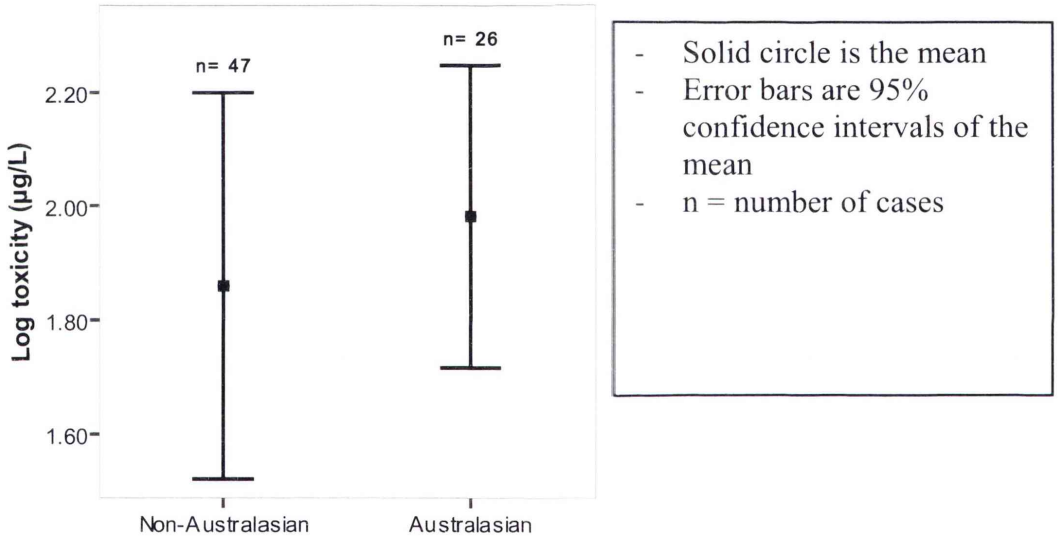

Figure 4.1 Explanation of the figures used throughout the rest of this chapter.

The comparisons between Australasian and non-Australasian taxa that were performed and the type of data that was used (e.g. EC/LC_{50}, LC_{50}, EC_{50}, HM = hardness modified), are listed in Table 4.1 for freshwater and Table 4.2 for marine/estuarine water. Hardness modification calculations can be found in the general method sections (Section 2.3).

Table 4.1 Comparisons that were performed between Australasian and non-Australasian freshwater taxa in this chapter and the type of data that was used

Metal/metalloid	Taxon	Toxicity data used			
		EC/LC_{50}	LC_{50}	EC_{50}	HM
As (V)	Chordata	Y	Y	X	X
	Chlorophyta	X	X	Y	X
	Uniramia	Y	Y	X	X
Cd	Chlorophyta	Y	X	X	X
	Chordata	Y	Y	X	X
	Crustacea	Y	Y	Y	X
	Magnoliophyta	Y	X	X	X
	Mollusca	Y	X	X	X
	Uniramia	Y	Y	X	X
Cr (VI)	Chlorophyta	Y	X	Y	X
	Crustacea	Y	X	Y	X
Cu	Chlorophyta	Y	X	Y	Y
	Chordata	Y	Y	X	Y
	Crustacea	Y	Y	Y	Y
	Mollusca	Y	Y	X	Y
	C. robustus & *C. destructor*	Y	X	X	Y
	C. dubia & *C.* cf. *dubia*	Y	X	X	Y
	D. pulex & *C.* cf. *dubia*	Y	X	X	Y
	D. magna & *C.* cf. *dubia*	Y	X	X	Y
	M. rosenbergi & *P. australiensis*	Y	X	X	Y
	P. subcapitata & *Chlorella* sp.	Y	X	X	X
	S. quadricauda & *P. subcapitata*	Y	X	X	X
	S. quadricauda & *Chlorella* sp.	Y	X	X	X
	P. promelas & *M. splendida inornata*	X	X	X	Y
	P. subcapitata (Non-A) & *P. subcapitata* (Aust)	Y	X	X	X
	V. iris & *H. depressa*	Y	X	X	Y
Pb	Chordata	Y	Y	X	Y
Hg	Chordata	Y	Y	X	X
	Crustacea	Y	Y	X	X
U	Chordata	Y	X	X	X
	Crustacea	Y	Y	X	X
	D. magna & *M. macleayi*	Y	X	X	X
Zn	Chlorophyta	Y	X	Y	X
	Chordata	Y	Y	X	Y
	Crustacea	Y	Y	X	Y
	D. magna & *C.* cf. *dubia*	Y	X	X	Y

(HM is hardness modified data i.e. the data have been standardised to a hardness of 30 mg/L $CaCO_3$). Y indicates the comparison could be made while X indicates it could not due to small sample size ($n < 3$)

Table 4.2 Comparisons that were performed between Australasian and non-Australasian marine/estuarine taxa and the type of data used

Metal	Taxon	Toxicity data used		
		EC/LC_{50}	LC_{50}	EC_{50}
Cd	Chordata	X	Y	X
	Crustacea	Y	Y	X
	Echinodermata	X	X	Y
	Mollusca	Y	X	X
Cu	Chordata	Y	Y	X
	Crustacea	Y	Y	X
	Echinodermata	Y	X	X
	Mollusca	Y	Y	Y
	P. japonicus & *P. merguensis*	Y	X	X
Pb	Crustacea	Y	Y	X
Ni	Crustacea	X	Y	X
Zn	Crustacea	Y	Y	X
	Mollusca	Y	Y	Y

Y indicates the comparison could be made while X indicates it could not due to small sample size ($n < 3$).

4.3 RESULTS

Results for comparisons undertaken using 95% confidence intervals and Student's t-test are given in Tables 4-3 to 4-9. The comparisons that did not have overlapping 95% CIs (and are therefore significantly different) do not have values for the t-test, degrees of freedom, significance or power in cells in Tables 4-3 to 4-9. The difference in toxicity noted in each description of results was calculated by converting the log toxicity data back to toxicity data and then deriving the difference in sensitivity with these values.

Only those comparisons where significant differences were detected between non-Australasian and Australasian taxa are described and the results graphically presented in the results sections (e.g. Figures 4.2 to 4.37). The comparisons where no significant differences were detected are displayed in Appendix 5. Tests undertaken using a Mann-Whitney have been denoted by a U in parentheses in the t value column.

4.3.1 Freshwater comparisons using all data (LC_{50} and EC_{50})

Table 4.3 Freshwater results for 95% confidence interval and Student's t-test for all data

Metal/ Metalloid	Taxa	Mean (95% CI) Non-A	Aust	t value	Degrees of Freedom	Significance	Power
As (V)	Chordata	3.86 (3.54 – 4.17)	3.81 (3.17-4.45)	0.118	77	0.909	0.052
	Uniramia	4.47 (4.04-4.89)	5.40 (5.19-5.60)				
Cd	Chlorophyta	1.80 (1.52-2.07)	1.45 (1.02-1.89)	1.216	28	0.234	0.358
	Chordata	3.38 (3.00-3.76)	3.19 (2.67-3.71)	0.5	66	0.619	0.094
	Crustacea	1.86 (1.52-2.20)	1.98 (1.72-2.25)	-0.569	70.9	0.571	0.087
	Magnoliophyta	2.02 (1.73-2.31)	3.08 (1.83-4.33)	-2.253	4.63	0.078	0.618
	Mollusca	2.57 (2.10-3.05)	2.33 (2.07-2.59)	142 (U)	34	0.634	0.150
	Uniramia	4.22 (3.56-4.88)	3.69 (2.05-5.33)	0.825	20	0.419	0.116
	P. subcapitata (Non-A) & *P. subcapitata* (Aust)	1.69 (1.36-2.02)	1.45 (1.02-1.89)	0.93	18	0.365	0.185
Cr (VI)	Chlorophyta	2.75 (2.52-2.97)	2.22 (1.80-2.64)	2.538	19	0.020	0.822
	Crustacea	2.36 (2.14-2.59)	1.86 (1.52-2.19)	2.340	61	0.023	0.746
Cu	Chlorophyta	2.27 (2.01-2.53)	0.92 (0.77-1.07)				
	Chordata	1.96 (1.78-2.14)	2.06 (1.92-2.20)	-0.870	165.4	0.386	0.141
	Crustacea	1.93 (1.75-2.12)	1.86 (1.65-2.08)	0.502	115	0.616	0.078
	Mollusca	1.51 (1.38-1.64)	2.13 (1.66-2.60)				
	C. robustus & *C. destructor*	2.90 (2.66-3.13)	3.30 (2.71-3.88)	-1.877	15	0.080	0.395
	C. dubia & *C.* cf. *dubia*	1.33 (0.82-1.83)	0.86 (0.31-1.41)	1.456	14	0.167	0.318
	D. pulex & *C.* cf. *dubia*	1.52 (1.41-1.64)	0.86 (0.31-1.41)				
	D. magna & *C.* cf *dubia*	1.33 (0.99-1.68)	0.86 (0.31-1.41)	1.680	11.4	0.120	0.390
	M. rosenbergi & *P. australiensis*	2.02 (1.82-2.22)	2.05 (1.94-2.16)	-0.293	32	0.771	0.062
	P. subcapitata (Non-A) & *P. subcapitata* (Aust)	1.67 (1.42-1.92)	1.35 (0.90-1.80)	1.528	18	0.144	0.268
	S. quadricauda & *P. subcapitata*	2.45 (2.20-2.70)	1.35 (0.90-1.80)				
	S. quadricauda & *Chlorella* sp.	2.45 (2.20-2.70)	0.79 (0.62-0.95)	1 (U)	31	0.000	0.990
	V. iris & *H. depressa*	1.72 (1.63-1.81)	1.90 (1.48-2.31)	-0.899	10.9	0.388	0.147
Pb	Chordata	3.97 (3.64-4.30)	3.69 (3.03-4.36)	0.893	25	0.380	0.144

Metal/ Metalloid	Taxa	Mean (95% CI) Non-A	Aust	t value	Degrees of Freedom	Significance	Power
Hg	Chordata	2.91 (2.73-3.08)	1.61 (1.12-2.09)				
	Crustacea	1.60 (1.11-2.09)	0.70 (-0.26-1.66)	1.928	20	0.068	0.580
U	Chordata	4.55 (4.32-4.79)	3.34 (3.27-3.42)				
	Crustacea	4.27 (3.84-4.69)	2.57 (2.12-3.01)				
	D. magna & *M. macleayi*	4.27 (3.84-4.69)	2.34 (1.66-3.02)	6.643	10	0.035	0.990
Zn	Chlorophyta	2.23 (1.86-2.60)	2.27 (1.61-2.93)	-0.127	24	0.900	0.051
	Chordata	3.83 (3.67-3.99)	3.48 (3.15-3.81)	1.693	127	0.093	0.509
	Crustacea	3.37 (2.95-3.78)	3.00 (2.65-3.34)	1.441	45	0.157	0.304
	D. magna & *C.* cf. *dubia*	2.77 (2.16-3.38)	2.40 (1.99-2.81)	1.296	11	0.221	0.240

4.3.2 Freshwater comparisons using LC_{50} data

Table 4.4 Freshwater results for 95% confidence interval and Student's t-test for LC_{50} data

Metal/ Metalloid	Taxa	Mean (95% CI) Non-A	Aust	t value	Degrees of Freedom	Significance ($p < 0.05$)	Power
As (V)	Chordata	3.86 (3.54-4.17)	4.06 (3.40-4.72)	-0.450	75	0.654	0.094
	Uniramia	4.52 (4.03-5.00)	5.40 (5.19-5.60)				
Cd	Chordata	3.39 (3.00-3.78)	3.19 (2.67-3.71)	0.647	33.9	0.522	0.099
	Crustacea	2.12 (1.78-2.36)	2.07 (1.78-2.36)	0.187	58	0.853	0.055
	Uniramia	4.23 (3.46-5.00)	4.43 (2.25-6.61)	-0.267	16	0.793	0.058
Cu	Chordata	1.97 (1.78-2.15)	2.06 (1.92-2.20)	-0.787	161.7	0.432	0.124
	Crustacea	1.91 (1.70-2.11)	2.01 (1.81-2.21)	-0.735	98	0.464	0.111
	Mollusca	1.51 (1.38-1.64)	3.04 (1.60-4.47)	33 (U)	52	0.001	0.775
Pb	Chordata	3.97 (3.64-4.30)	3.69 (3.03-4.36)	0.893	25	0.380	0.144
Hg	Chordata	2.91 (2.73-3.08)	1.61 (1.12-2.09)				
	Crustacea	1.60 (1.11-2.09)	0.70 (-0.26-1.66)	1.928	20	0.068	0.580
U	Crustacea	4.27 (3.84-4.69)	2.57 (2.12-3.01)				
Zn	Chordata	3.84 (3.68-4.00)	3.48 (3.15-3.81)	1.729	123	0.087	0.527
	Crustacea	3.38 (2.92-3.84)	3.20 (2.81-3.59)	0.624	37	0.537	0.095

4.3.3 Freshwater comparisons using EC_{50} data

Table 4.5 Freshwater results for 95% confidence intervals and Student's t-test for EC_{50} data

Metal/ Metalloid	Taxa	Mean (95% CI) Non-A	Aust	t value	Degrees of Freedom	Significance ($p < 0.05$)	Power
As (V)	Chlorophyta	2.04 (1.61-2.48)	3.01 (1.34-4.69)	-1.916	21	0.069	0.407
Cd	Crustacea	0.79 (-0.11-1.68)	1.49 (0.72-2.27)	-1.153	11	0.274	0.339
Cr (VI)	Chlorophyta	2.76 (2.50-3.02)	2.22 (1.80-2.64)	2.454	17	0.025	0.809
	Crustacea	2.06 (1.87-2.25)	1.95 (1.59-2.30)	0.658	35	0.515	0.094
Cu	Chlorophyta	2.27 (2.01-2.53)	0.92 (0.77-1.07)				
	Crustacea	2.07 (1.58-2.55)	0.72 (0.13-1.31)				
Zn	Chlorophyta	2.23 (1.86-2.60)	2.27 (1.61-2.93)	-0.127	24	0.900	0.052

4.3.4 Freshwater comparisons using hardness modified data

Table 4.6 Freshwater results for 95% confidence intervals and Student's t-test for hardness modified data

Metal/ Metalloid	Taxa	Mean (95% CI) Non-A	Aust	t value	Degrees of Freedom	Significance	Power
Cu	Chordata	2.20 (1.96-2.44)	2.06 (1.88-2.24)	0.933	165.5	0.352	0.154
	Chlorophyta	1.67 (0.68-2.66)	1.16 (0.92-1.40)	1.391	37	0.173	0.233
	Crustacea	2.12 (1.93-2.31)	1.73 (1.51-1.96)	2.620	111	0.010	0.750
	Mollusca	2.08 (1.95-2.21)	2.26 (1.70-2.83)	-0.675	64	0.346	0.100
	C. robusta & *C. destructor*	2.53 (2.29-2.76)	3.66 (3.05-4.27)				
	C. dubia & *C.* cf. *dubia*	1.47 (0.70-2.24)	1.09 (0.48-1.70)	18 (U)	14	0.174	0.150
	D. pulex & *C.* cf. *dubia*	2.05 (1.89-2.21)	1.09 (0.48-1.70)				
	D. magna & *C.* cf. *dubia*	1.61 (0.68-2.54)	1.09 (0.48-1.70)	9 (U)	11	0.250	0.220
	M. rosenbergi & *P. australiensis*	2.19 (1.99-2.39)	1.81 (1.70-1.92)				
	P. promelas & *M. splendida*	1.21 (0.91-1.51)	2.47 (2.08-2.87)				
	V. iris & *H. depressa*	2.28 (2.12-2.43)	1.98 (1.57-2.40)	1.466	12.7	0.167	0.330
Pb	Chordata	4.27 (3.67-4.87)	3.19 (2.38-4.00)	1.940	24	0.064	0.720
Zn	Chordata	4.29 (4.09-4.50)	3.40 (3.04-3.77)				
	Crustacea	3.63 (2.80-4.46)	2.65 (2.56-3.33	1.836	32	0.076	0.370
	D. magna & *C.* cf. *dubia*	3.07 (1.77-4.37)	2.64 (2.26-3.02)	1.181	9	0.268	0.170

4.3.5 Marine/estuarine comparisons using all data (LC_{50} and EC_{50})

Table 4.7 Marine/estuarine results for 95% confidence interval and Student's t-test for all data

Metal/ Metalloid	Taxa	Mean diff Non-A	Aust	t value	Degrees of Freedom	Significance ($p < 0.05$)	Power
Cd	Crustacea	2.54 (2.31-2.76)	3.13 (2.86-3.40)				
	Mollusca	3.33 (2.91-3.74)	3.08 (2.67-3.48)	0.833	19	0.415	0.160
Cu	Chordata	1.60 (0.81-2.39)	3.32 (2.91-3.74)				
	Crustacea	2.53 (2.26-2.81)	2.46 (2.26-2.66)	0.405	114.5	0.686	0.070
	Echinodermata	1.65 (1.47-1.82)	1.34 (1.08-1.60)	2.163	17.4	0.045	0.590
	Mollusca	1.78 (1.46-2.09)	1.86 (1.40-2.33)	-0.337	36	0.738	0.060
	P. japonicus & *P. merguensis*	1.86 (1.44-2.28)	2.82 (2.39-3.26)				
Pb	Crustacea	4.64 (3.24-6.03)	4.41 (3.85-4.97)	0.368	8.03	0.722	0.066
Zn	Crustacea	2.76 (2.49-3.03)	3.10 (2.93-3.27)	-2.160	73.8	0.034	0.580
	Mollusca	2.92 (2.49-3.35)	2.94 (2.17-3.70)	-0.370	23	0.971	0.050

4.3.6 Marine/estuarine comparisons using LC_{50} data

Table 4.8 Marine/estuarine results for 95% confidence intervals and Student's t-test for LC_{50} data

Metal/ Metalloid	Taxa	Mean (95% CI) Non-A	Aust	t value	Degrees of Freedom	Significance ($p < 0.05$)	Power
Cd	Chordata	2.95 (2.05-3.85)	3.93 (3.70-4.15)	-0.957	27	0.347	0.110
	Crustacea	2.56 (2.33-2.80)	3.25 (3.00-3.50)				
Cu	Chordata	1.43 (0.39-2.48)	3.32 (2.91-3.74)				
	Crustacea	2.55 (2.27-2.83)	2.56 (2.36-2.75)	-0.170	107.3	0.985	0.050
	Mollusca	2.03 (1.66-2.41)	2.49 (2.06-2.91)	-1.421	21	0.170	0.460
Pb	Crustacea	4.92 (2.75-7.10)	4.41 (3.85-4.97)	0.629	4.7	0.569	0.100
Ni	Crustacea	3.12 (2.43-3.82)	3.80 (3.42-4.18)	-2.185	15	0.045	0.540
Zn	Crustacea	2.76 (2.49-3.03)	3.15 (3.00-3.31)	-2.546	71.1	0.013	0.710
	Mollusca	3.29 (2.97-3.62)	3.76 (3.38-4.13)	-1.615	15	0.127	0.660

4.3.7 Marine/estuarine comparisons using EC_{50} data

Table 4.9 Marine/estuarine results for 95% confidence intervals and Student's t-test for EC_{50} data

Metal/ Metalloid	Taxa	Mean (95% CI) Non-A	Aust	t value	Degrees of Freedom	Significance ($p < 0.05$)	Power
Cd	Echinodermata	3.36 (2.70-4.01)	2.87 (2.28-3.46)	1.476	8	0.178	0.410
Cu	Mollusca	1.22 (0.80-1.65)	1.33 (0.79-1.87)	21 (U)	13	0.463	0.064
Zn	Mollusca	1.71 (1.50-1.92)	2.12 (1.62-2.61)	-2.396	6	0.054	0.482

4.3.8 Comparisons of freshwater taxa

4.3.8.1 Log toxicity (LC_{50} and EC_{50} combined) of arsenic (V) to Uniramia in freshwater

A summary of the statistics for the log toxicity of As (V) to freshwater Uniramia is presented in Figure 4.2. The mean log toxicity and 95% confidence intervals of As (V) to these Australasian and non-Australasian species are 5.40 (5.19 - 5.60) and 4.47 (4.04 - 4.89) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian Uniramia being approximately seven times more sensitive.

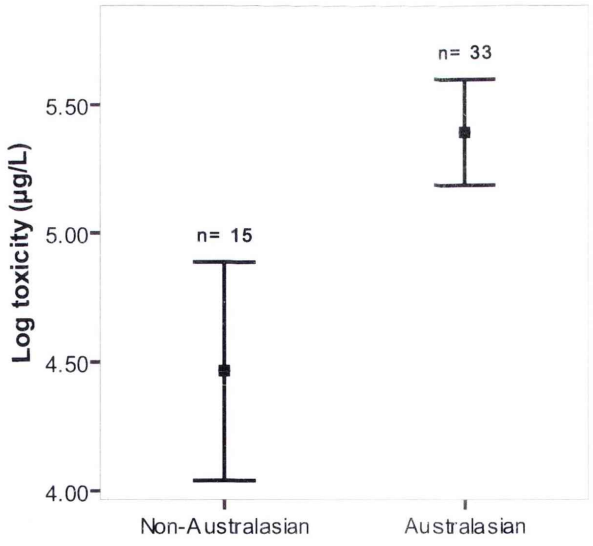

Figure 4.2 Log toxicity of arsenic (V) to non-Australasian and Australasian Uniramia.

4.3.8.2 Log toxicity (LC50 and EC50 combined) of chromium (VI) to Chlorophyta in freshwater

A summary of the statistics for the log toxicity of Cr (VI) to freshwater Chlorophyta is presented in Figure 4.3. The mean log toxicity and 95% confidence intervals of Cr (VI) to these Australasian and non-Australasian species are 2.22 (1.80 - 2.64) and 2.75 (2.52 - 2.97) respectively. A significant difference ($t = 2.54$, $df = 19$, $p = 0.02$) was detected between the two sets of organisms using a Student's t-test. The Australasian freshwater Chlorophyta was approximately three times more sensitive to Cr (VI) the non-Australasian Chlorophyta.

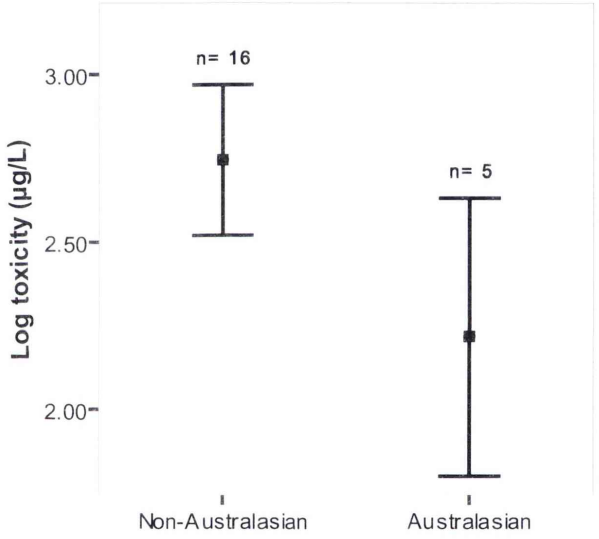

Figure 4.3 Log toxicity of chromium (VI) to non-Australasian and Australasian Chlorophyta.

4.3.8.3 Log toxicity (LC_{50} and EC_{50} combined) of chromium (VI) to Crustacea in freshwater

A summary of the statistics for the log toxicity of Cr (VI) to freshwater Crustaceans is presented in Figure 4.4. The mean log toxicity and 95% confidence intervals of Cr (VI) to these Australasian and non-Australasian species are 1.86 (1.52 - 2.19) and 2.36 (2.14 - 2.59) respectively. A significant difference ($t = 2.34$, $df = 61$, $p = 0.023$) was detected between the two sets of organisms using a Student's t-test. The Australasian freshwater Crustacea was approximately three times more sensitive to Cr (VI) the non-Australasian Crustacea.

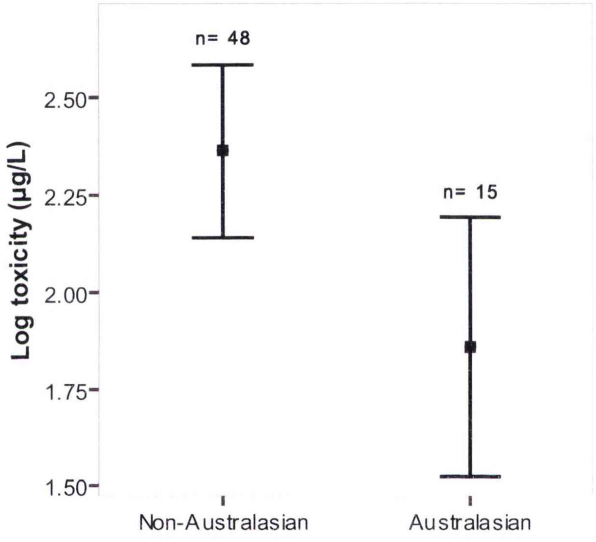

Figure 4.4 Log toxicity of chromium (VI) to non-Australasian and Australasian Crustacea.

4.3.8.4 Log toxicity (LC_{50} and EC_{50} combined) of copper to Chlorophyta in freshwater

A summary of the statistics for the log toxicity of Cu to freshwater Chlorophyta is presented in Figure 4.5. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 0.92 (0.77 - 1.07) and 2.27 (2.01 - 2.53) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different, with the Australasian Chlorophyta being approximately 22 times more sensitive.

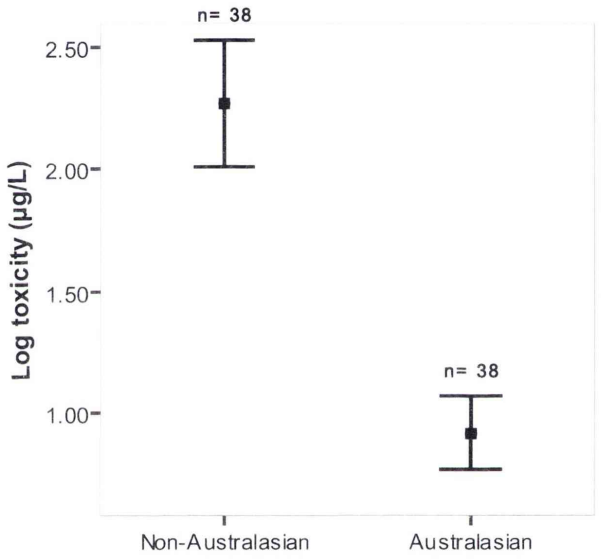

Figure 4.5 Log toxicity of copper to non-Australasian and Australasian Chlorophyta.

4.3.8.5 Log toxicity (LC_{50} and EC_{50} combined) of copper to Mollusca in freshwater

A summary of the statistics for the log toxicity of Cu to freshwater Mollusca is presented in Figure 4.6. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 2.13 (1.66 - 2.60) and 1.51 (1.38 - 1.64) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different, with the non-Australasian Mollusca being approximately four times more sensitive.

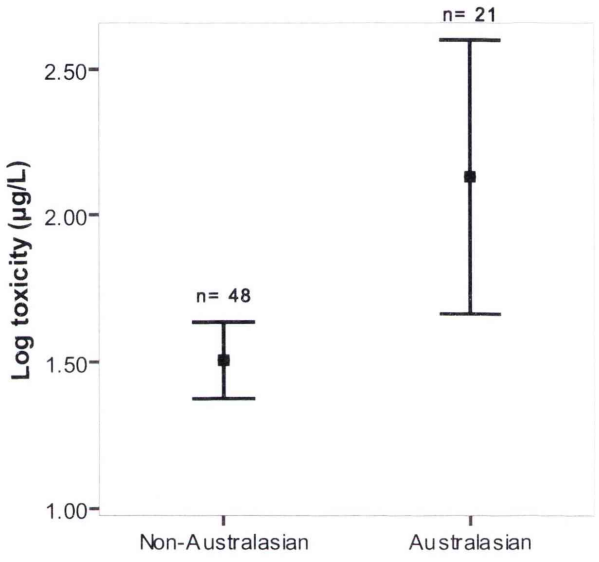

Figure 4.6 Log toxicity of copper to non-Australasian and Australasian Mollusca.

4.3.8.6 Toxicity (LC_{50} and EC_{50} combined) of copper to the cladocera *D. pulex* and *C.* cf. *dubia* in freshwater

A summary of the statistics for the toxicity of Cu to *D. pulex* and *C.* cf. *dubia* is presented in Figure 4.7. The mean toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 13.17 (2.4 – 23.94) and 1.52 (1.41 - 1.64) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different, with the Australasian waterflea, *C.* cf *dubia*, approximately five times more sensitive than the non-Australasian waterflea, *D. pulex*.

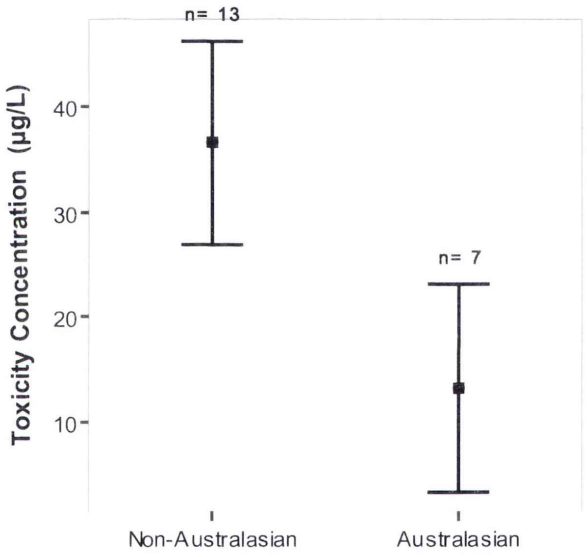

Figure 4.7 Toxicity of copper to the cladocera *D. pulex* and *C.*cf. *dubia*.

4.3.8.7 Log toxicity (LC_{50} and EC_{50} combined) of copper to the algae *S. quadricauda* and *P. subcapitata* in freshwater

A summary of the statistics for the log toxicity of Cu to *S. quadricauda* and *P. subcapitata* is presented in Figure 4.8. The mean log toxicity and 95% confidence intervals of Cu to the Australasian and non-Australasian species are 1.35 (0.90 - 1.80) and 2.45 (2.20 - 2.70) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different, with the Australasian *P. subcapitata* approximately 13 times more sensitive.

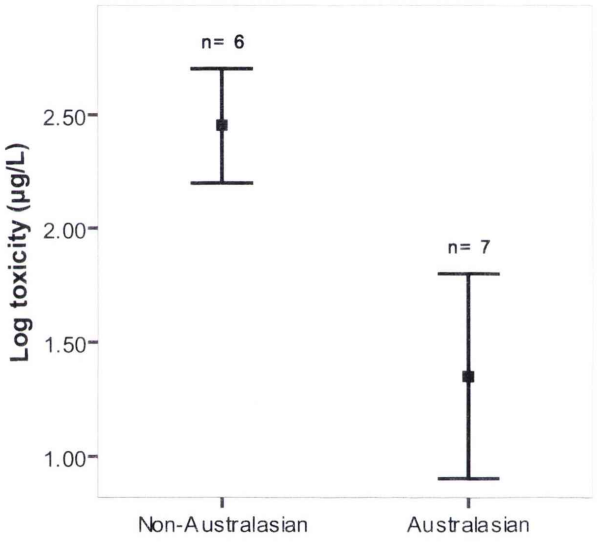

Figure 4.8 Log toxicity of copper to *S. quadricauda* and *P. subcapitata*.

4.3.8.8 Log toxicity (LC_{50} and EC_{50} combined) of copper to *S. quadricauda* and *Chlorella* sp. in freshwater

A summary of the statistics for the log toxicity of Cu to *S. quadricauda* and *Chlorella* sp. is presented in Figure 4.9. The mean log toxicity and 95% confidence intervals of Cu to the Australasian and non-Australasian species are 0.79 (0.62 - 0.95) and 2.45 (2.20 - 2.70) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the Australasian *Chlorella* sp. being five times more sensitive.

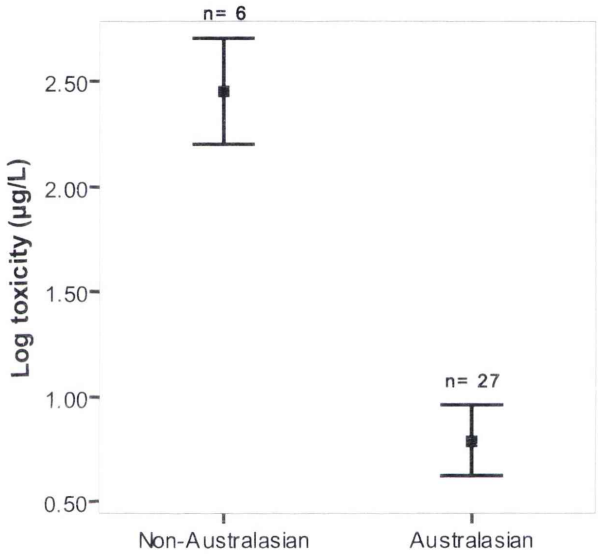

Figure 4.9 Log toxicity of copper to *S. quadricauda* and *Chlorella* sp.

4.3.8.9 Log toxicity (LC_{50} and EC_{50} combined) of copper to *P. promelas* and *M. splendida inornata* in freshwater

A summary of the statistics for the log toxicity of Cu to *P. promelas* and *M. splendida inornata* is presented in Figure 4.10. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 2.53 (2.28 - 2.78) and 1.36 (1.14 - 1.57) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian *P. promelas* being approximately 15 times more sensitive.

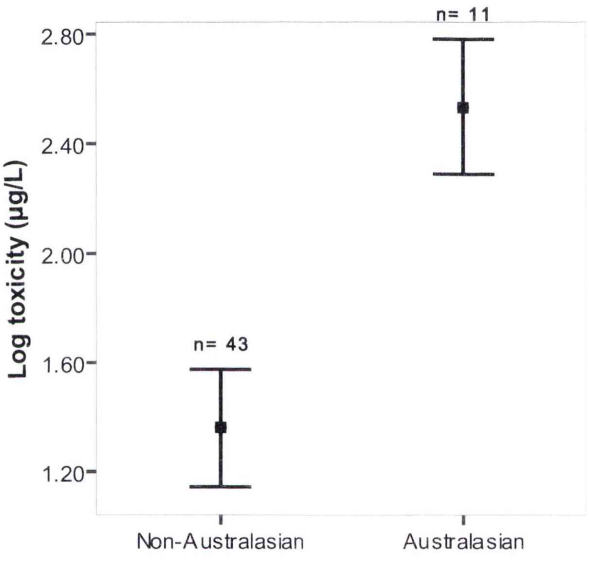

Figure 4.10 Log toxicity of copper to *P. promelas* and *M. splendida inornata*.

4.3.8.10 Log toxicity (LC_{50} and EC_{50} combined) of mercury to Chordata in freshwater

A summary of the statistics for the log toxicity of Hg to freshwater Chordata is presented in Figure 4.11. The mean log toxicity and 95% confidence intervals of Hg to these Australasian and non-Australasian species are 1.61 (1.12 - 2.09) and 2.91 (2.73 - 3.08) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different, with the Australasian Chordata being approximately 20 times more sensitive.

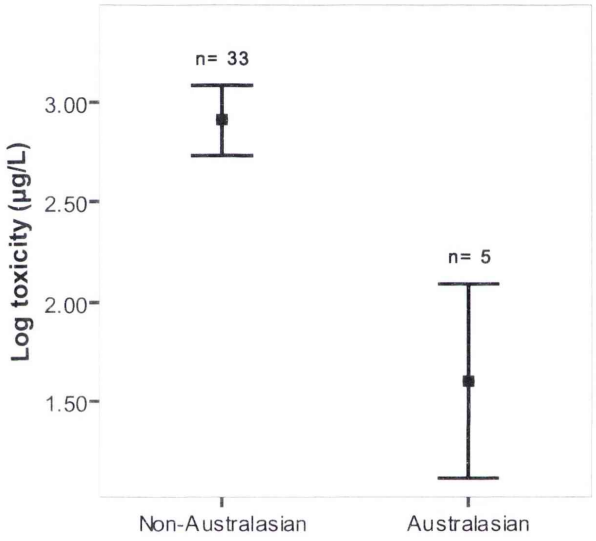

Figure 4.11 Log toxicity of mercury to non-Australasian and Australasian Chordata.

4.3.8.11 Log toxicity (LC_{50} and EC_{50} combined) of uranium to Chordata in freshwater

A summary of the statistics for the log toxicity of U to freshwater Chordata is presented in Figure 4.12. The mean log toxicity and 95% confidence intervals of U to these Australasian and non-Australasian species are 3.34 (3.27 - 3.42) and 4.55 (4.32 - 4.79) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different, with the Australasian Chordata being approximately 16 times more sensitive.

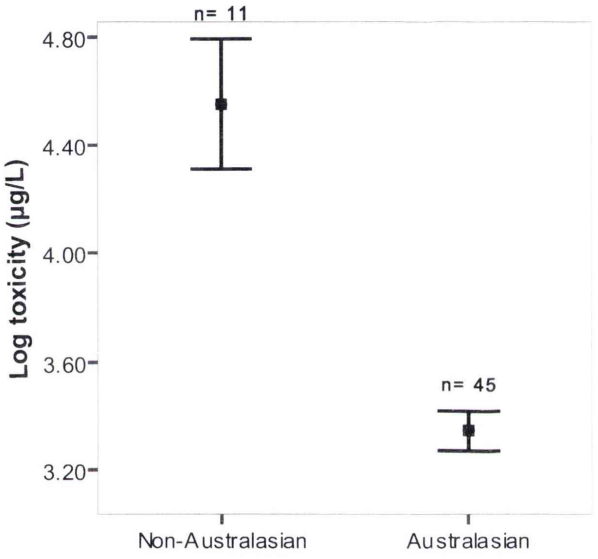

Figure 4.12 Log toxicity of uranium to non-Australasian and Australasian Chordata.

4.3.8.12 Log toxicity (LC_{50} and EC_{50} combined) of uranium to Crustacea in freshwater

A summary of the statistics for the log toxicity of U to freshwater Crustacea is presented in Figure 4.13. The mean log toxicity and 95% confidence intervals of U to these Australasian and non-Australasian species are 2.57 (2.12 - 3.01) and 4.27 (3.84 - 4.69) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different, with the Australasian Crustacea being approximately 50 times more sensitive.

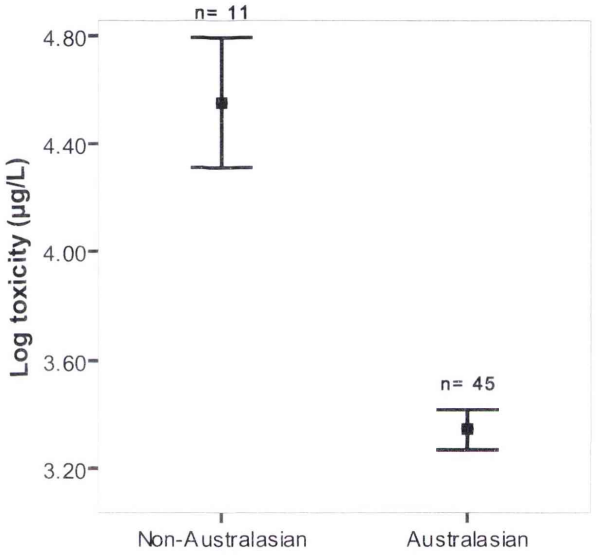

Figure 4.13 Log toxicity of uranium to non-Australasian and Australasian Crustacea.

4.3.8.13 Log toxicity (LC_{50} and EC_{50} combined) of uranium to *D. magna* and *M. macleayi* in freshwater

A summary of the statistics for the log toxicity of U to *D. magna* and *M. macleayi* is presented in Figure 4.14. The mean log toxicity and 95% confidence intervals of U to these Australasian and non-Australasian species are 2.34 (1.66 - 3.02) and 4.27 (3.84 - 4.69) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the Australasian *M. macleayi* being approximately 85 times more sensitive to uranium.

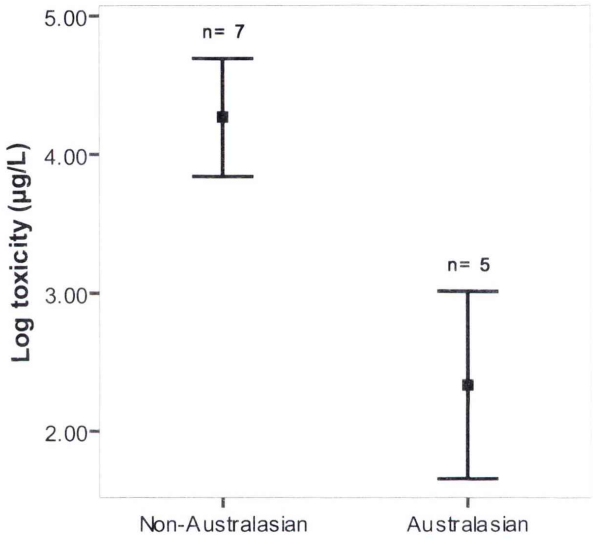

Figure 4.14 Log toxicity of uranium to *D. magna* and *M. macleayi*.

4.3.9 Comparisons of freshwater taxa – LC50 data only

4.3.9.1 Log toxicity (LC_{50}) of arsenic (V) to Uniramia in freshwater

A summary of the statistics for the LC_{50} values of As (V) to freshwater Uniramia is presented in Figure 4.15. The mean log toxicity and 95% confidence intervals of As (V) to these Australasian and non-Australasian species are 5.40 (5.19 - 5.60) and 4.52 (4.03 - 5.00) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian Uniramia being approximately eight times more sensitive.

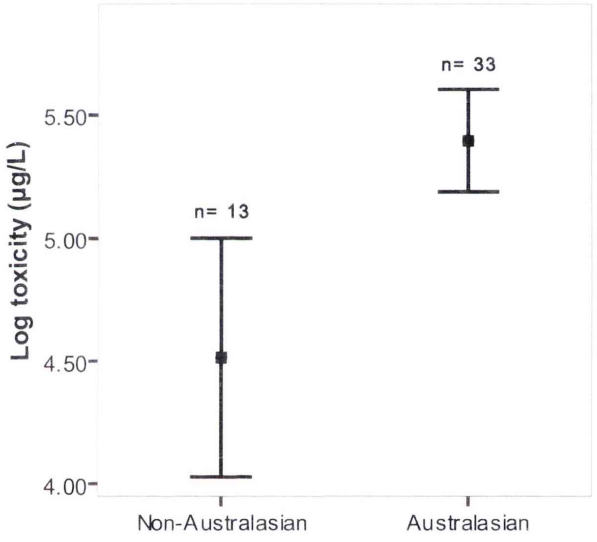

Figure 4.15 Log toxicity (LC_{50}) of arsenic (V) to non-Australasian and Australasian Uniramia.

4.3.9.2 Log toxicity (LC_{50}) of copper to Mollusca in freshwater

A summary of the statistics for the LC_{50} values of Cu to freshwater Mollusca is presented in Figure 4.16. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 3.04 (1.60 - 4.47) and 1.51 (1.38 - 1.64) respectively. A significant difference (U = 33, df = 52, p = 0.001) was detected between the two sets of organisms using a Mann-Whitney test. The Australasian freshwater Mollusca was approximately 34 times more sensitive to Cu the non-Australasian Mollusca.

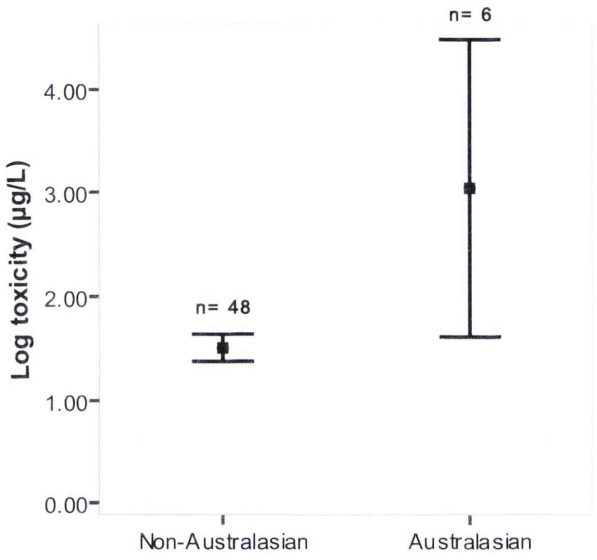

Figure 4.16 Log toxicity (LC_{50}) of copper to non-Australasian and Australasian Mollusca.

4.3.9.3 Log toxicity (LC_{50}) of mercury to Chordata in freshwater

A summary of the statistics for the LC_{50} values of Hg to freshwater Chordata is presented in Figure 4.17. The mean log toxicity and 95% confidence intervals of Hg to these Australasian and non-Australasian species are 1.61 (1.12 - 2.09) and 2.91 (2.73 - 3.08) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the Australasian Chordata being approximately 20 times more sensitive to Hg.

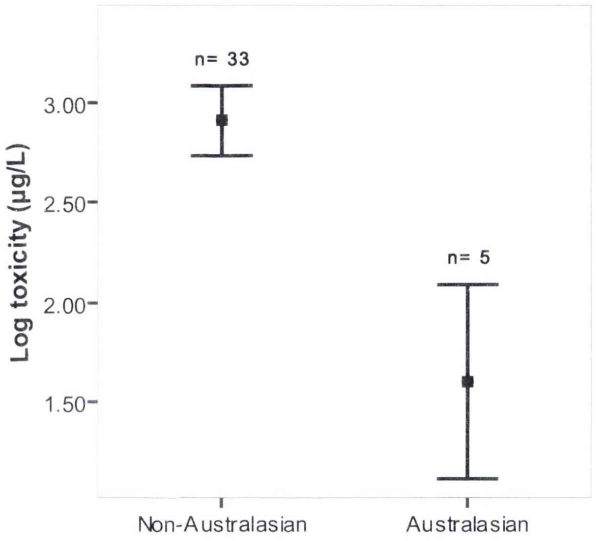

Figure 4.17 Log toxicity (LC_{50}) of mercury to non-Australasian and Australasian Chordata.

4.3.9.4 Log toxicity (LC_{50}) of uranium to Crustacea in freshwater

A summary of the statistics for the LC_{50} values of U to freshwater Crustacea is presented in Figure 4.18. The mean log toxicity and 95% confidence intervals of U to these Australasian and non-Australasian species are 2.57 (2.12 - 3.01) and 4.27 (3.84 - 4.69) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the Australasian Crustacea being approximately 50 times more sensitive to U.

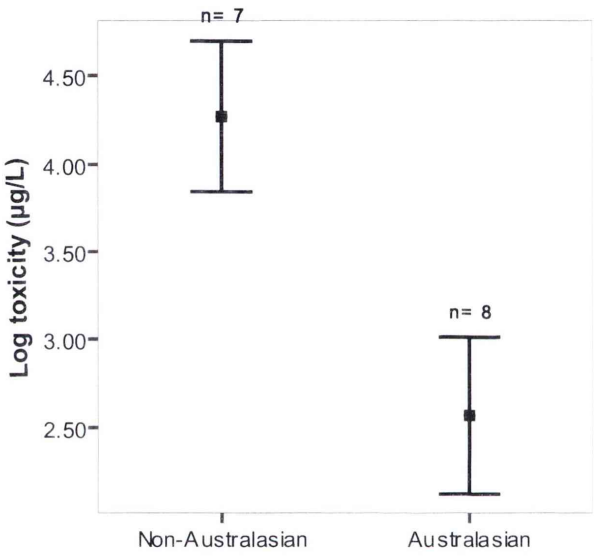

Figure 4.18 Log toxicity (LC_{50}) of uranium to non-Australasian and Australasian Crustacea.

4.3.10 Comparisons of freshwater taxa – EC_{50} data only

4.3.10.1 Log toxicity (EC_{50}) of chromium (VI) to Chlorophyta in freshwater

A summary of the statistics for the log toxicity of Cr (VI) to freshwater Chlorophyta is presented in Figure 4.19. The mean EC_{50} and 95% confidence intervals of Cr (VI) to these Australasian and non-Australasian species are 2.22 (1.80 - 2.64) and 2.76 (2.50 - 3.02) respectively. A significant difference ($t = 2.454$, $df = 17$, $p = 0.025$) was detected between the two sets of organisms based on a Student's t-test. The Australasian Chlorophyta are approximately twice as sensitive as the non-Australasian Chlorophyta.

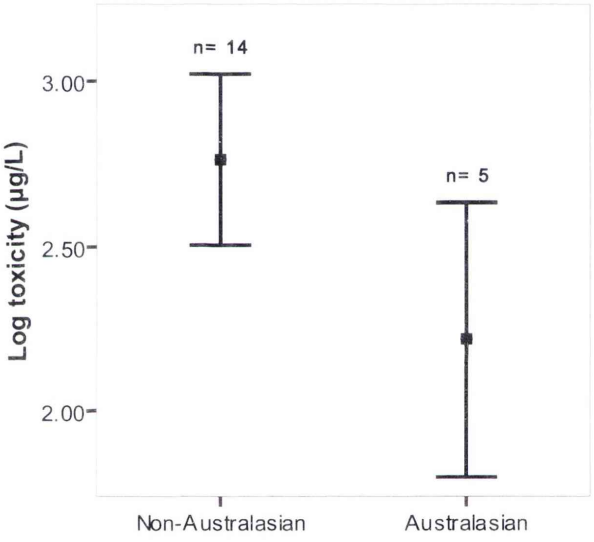

Figure 4.19 Log toxicity (EC_{50}) of chromium (VI) to non-Australasian and Australasian Chlorophyta.

4.3.10.2 Log toxicity (EC_{50}) of copper to Chlorophyta in freshwater

A summary of the statistics for the log toxicity of Cu to freshwater Chlorophyta is presented in Figure 4.20. The mean EC_{50} and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 0.92 (0.77 - 1.07) and 2.27 (2.01 - 2.53) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the Australasian Chlorophyta being approximately 22 times more sensitive.

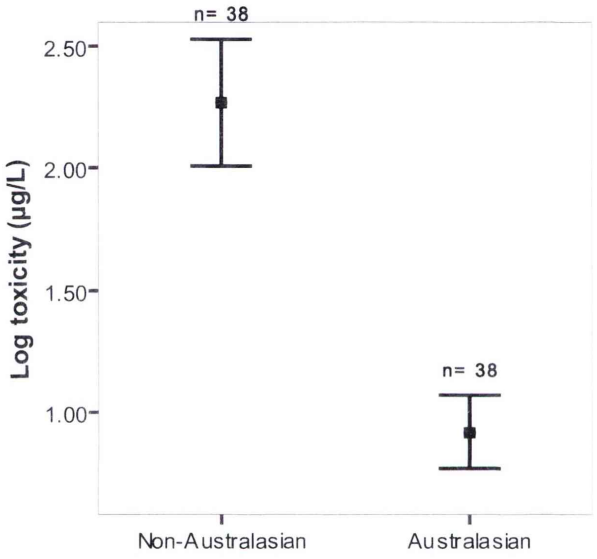

Figure 4.20 Log toxicity (EC_{50}) of copper to non-Australasian and Australasian Chlorophyta.

4.3.10.3 Log toxicity (EC_{50}) of copper to Crustacea in freshwater

A summary of the statistics for the log toxicity of Cu to freshwater Crustacea is presented in Figure 4.21. The mean EC_{50} and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 0.72 (0.13 - 1.31) and 2.07 (1.58 - 2.55) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the Australasian Crustacea being 22 times more sensitive.

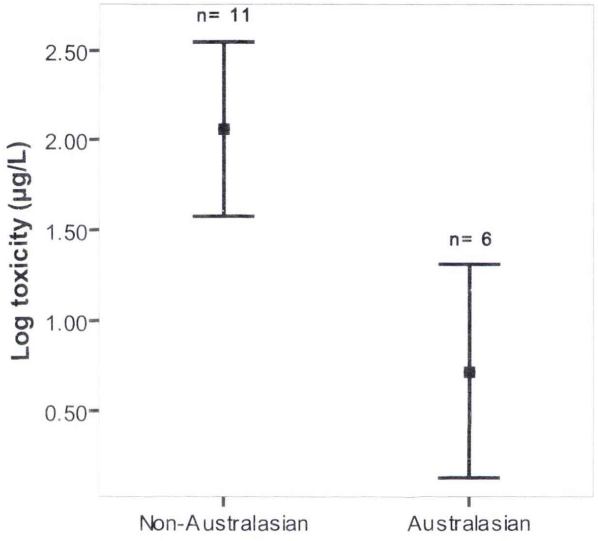

Figure 4.21 Log toxicity (EC_{50}) of copper to non-Australasian and Australasian Crustacea.

4.3.11 Comparisons of freshwater taxa – Hardness modified

4.3.11.1 Hardness modified log toxicity of copper to Crustacea in freshwater

A summary of the statistics for the hardness modified log toxicity of Cu to freshwater Crustacea is presented in Figure 4.22. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 1.73 (1.51 - 1.96) and 2.12 (1.93 - 2.31). A significant difference (t = 2.62, df = 111, p = 0.01) was detected between the two sets of organisms using a Student's t-test. The Australasian Crustacea are approximately twice as sensitive as the non-Australasian Crustacea.

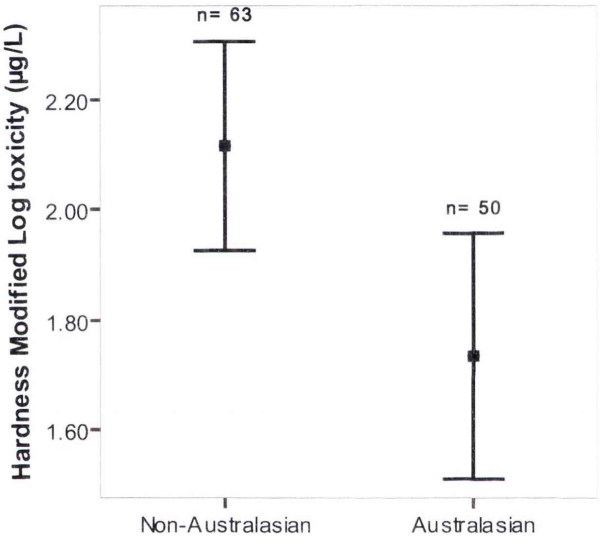

Figure 4.22 Log of the hardness modified toxicity data for copper to non-Australasian and Australasian Crustacea.

4.3.11.2 Hardness modified log toxicity of copper to the Crustaceans *C. robustus* and *C. destructor*

A summary of the statistics for the hardness modified log toxicity of Cu to *C. robustus* and *C. destructor* is presented in Figure 4.23. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 3.66 (3.05 - 4.27) and 2.53 (2.29 - 2.76) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian species are significantly different with the non-Australasian Crustacean being approximately 13 times more sensitive.

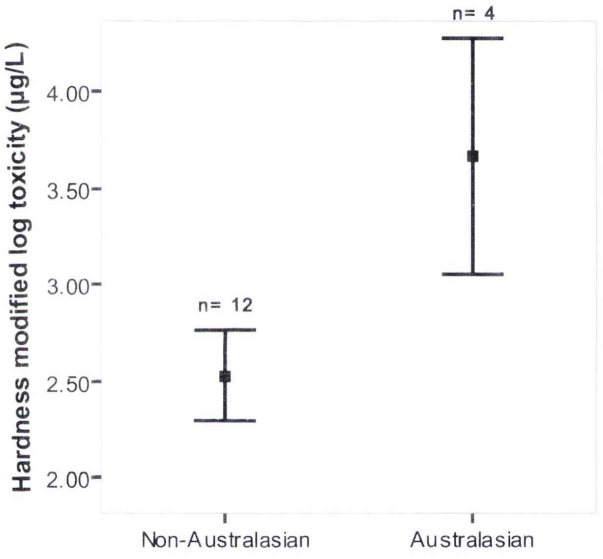

Figure 4.23 Log of the hardness modified toxicity data for copper to *C. robustus* and *C. destructor*.

4.3.11.3 Hardness modified log toxicity of copper to the crustaceans *D. pulex* and *C.* cf. *dubia*

A summary of the statistics for the hardness modified log toxicity of Cu to *D. pulex* and *C.* cf. *dubia* is presented in Figure 4.24. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 1.09 (0.48 - 1.69) and 2.05 (1.89 - 2.21) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian species are significantly different with the Australasian species being approximately nine times more sensitive.

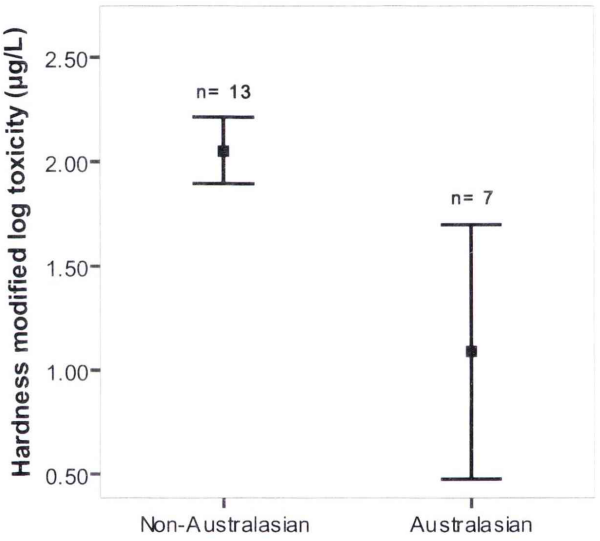

Figure 4.24 Log of the hardness modified toxicity data for copper to *D. pulex* and *C.* cf. *dubia*.

4.3.11.4 Hardness modified log toxicity of copper to the Crustaceans *M. rosenbergi* and *P. australiensis*

A summary of the statistics for the hardness modified log toxicity of Cu to *M. rosenbergi* and *P. australiensis* is presented in Figure 4.25. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 1.81 (1.70 - 1.92) and 2.19 (1.99 - 2.39) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the Australasian *P. australiensis* being approximately two times more sensitive.

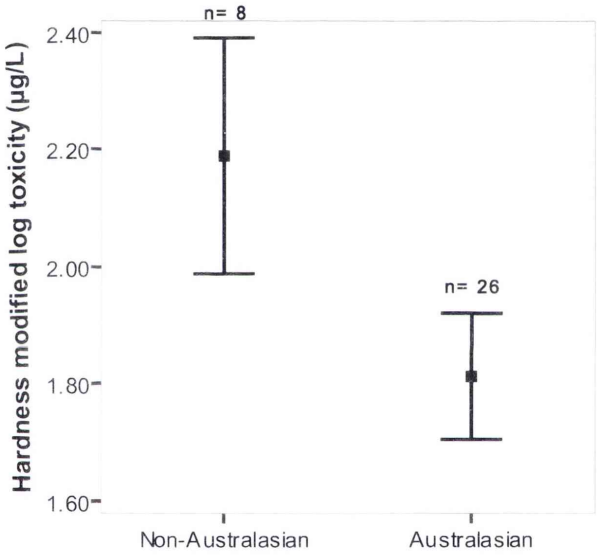

Figure 4.25 Log of the hardness modified toxicity data for copper to *M. rosenbergi* and *P. australiensis*.

4.3.11.5 Hardness modified log toxicity of copper to the Chordates *P. promelas* and *M. splendida inornata*

A summary of the statistics for the hardness modified log toxicity of Cu to *P. promelas* and *M. splendida inornata* is presented in Figure 4.26. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 2.47 (2.08 - 2.87) and 1.21 (0.91 - 1.51). As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian Chordata being approximately 18 times more sensitive.

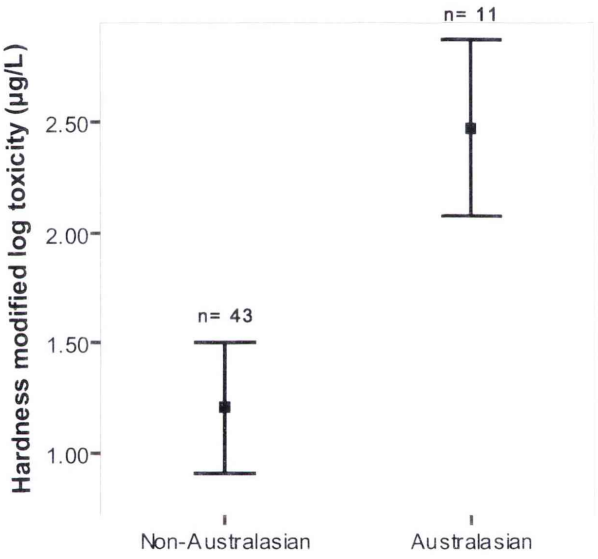

Figure 4.26 Log of the hardness modified log toxicity data for copper to *P. promelas* and *M. splendida inornata*.

4.3.11.6 Hardness modified log toxicity of zinc to Chordata in freshwater

A summary of the statistics for the hardness modified log toxicity of Zn to freshwater Chordata is presented in Figure 4.27. The mean log toxicity and 95% confidence intervals of Zn to these Australasian and non-Australasian species are 3.40 (3.04 - 3.77) and 4.29 (4.09 - 4.50) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the Australasian Chordata being approximately seven times more sensitive to Zn.

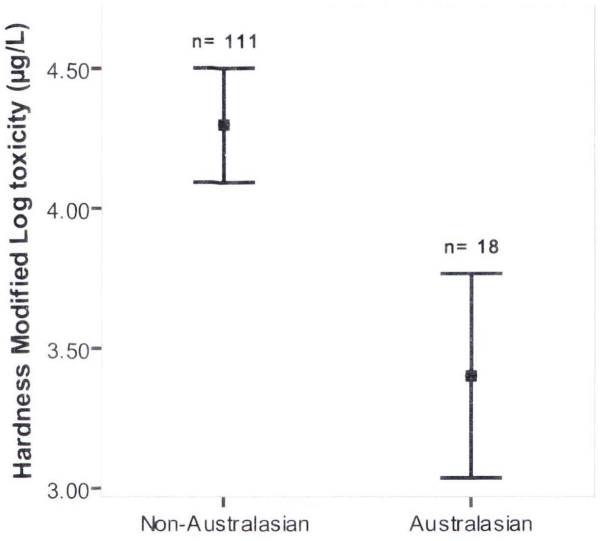

Figure 4.27 Log of the hardness modified toxicity data for zinc to non-Australasian and Australasian Chordata.

4.3.12 Comparisons of marine/estuarine taxa – EC_{50} and LC_{50} combined

4.3.12.1 Log toxicity of cadmium to Crustacea in marine/estuarine water

A summary of the statistics for the log toxicity of Cd to marine/estuarine Crustacea is presented in Figure 4.28. The mean log toxicity and 95% confidence intervals of Cd to these Australasian and non-Australasian species are 3.13 (2.86 - 3.40) and 2.54 (2.31 - 2.76) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian Crustacea being approximately four times more sensitive to Cd.

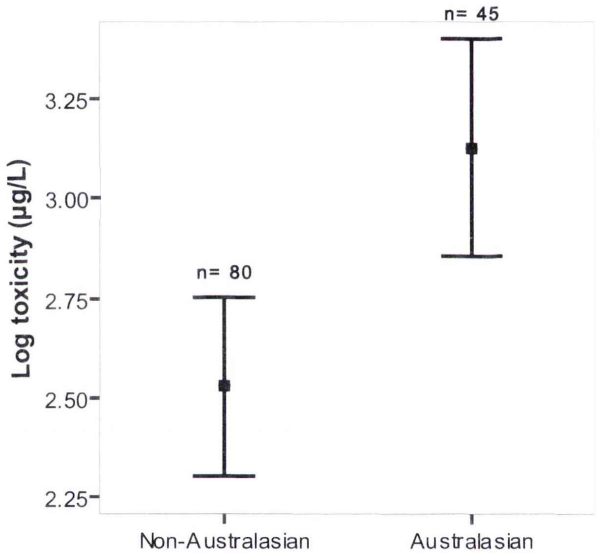

Figure 4.28 Log toxicity of cadmium to non-Australasian and Australasian Crustacea.

4.3.12.2 Log toxicity of copper to Chordata in marine/estuarine water

A summary of the statistics for the log toxicity of Cu to marine/estuarine Chordata is presented in Figure 4.29. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 3.32 (2.91 - 3.74) and 1.60 (0.81 - 2.39) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian Chordata being approximately 54 times more sensitive to Cu.

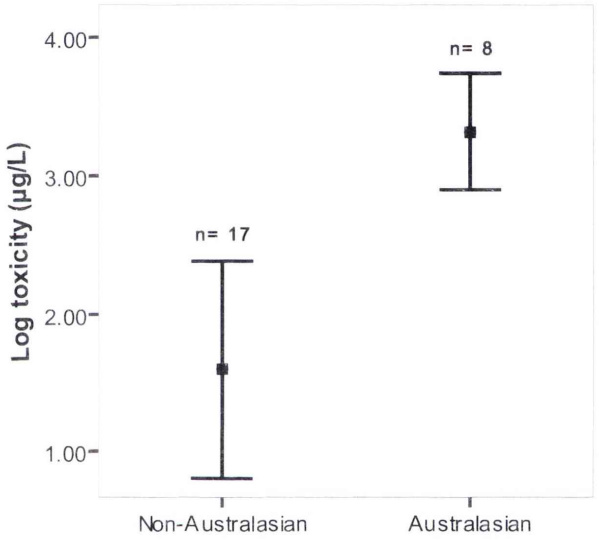

Figure 4.29 Log toxicity of copper to non-Australasian and Australasian Chordata.

4.3.12.3 Log toxicity of copper to Echinodermata in marine/estuarine water

A summary of the statistics for the log toxicity of Cu to marine/estuarine Echinodermata is presented in Figure 4.30. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 1.34 (1.08 - 1.60) and 1.65 (1.47 - 1.82). A significant difference (t = 2.163, df 17.4, p = 0.045, mod t-test) was detected between the two sets of organisms using a Student's t-test with the Australasian Echinodermata being approximately 1.4 times more sensitive.

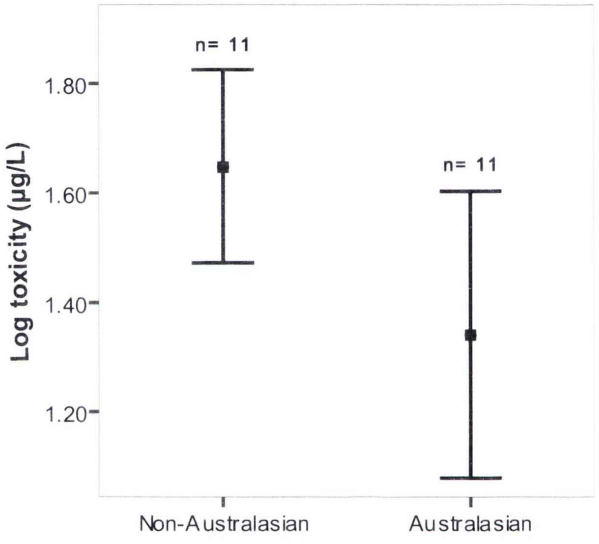

Figure 4.30 Log toxicity of copper to non-Australasian and Australasian Echinodermata.

4.3.12.4 Log toxicity of copper to the Crustaceans *P. japonicus* and *P. merguensis* in marine/estuarine water

A summary of the statistics for the log toxicity of Cu to *P. japonicus* and *P. merguensis* is presented in Figure 4.31. The mean log toxicity and 95% confidence intervals of Cu to the Australasian and non-Australasian species are 2.82 (2.39 - 3.26) and 1.86 (1.44 - 2.28) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian *P. merguensis* approximately nine times more sensitive.

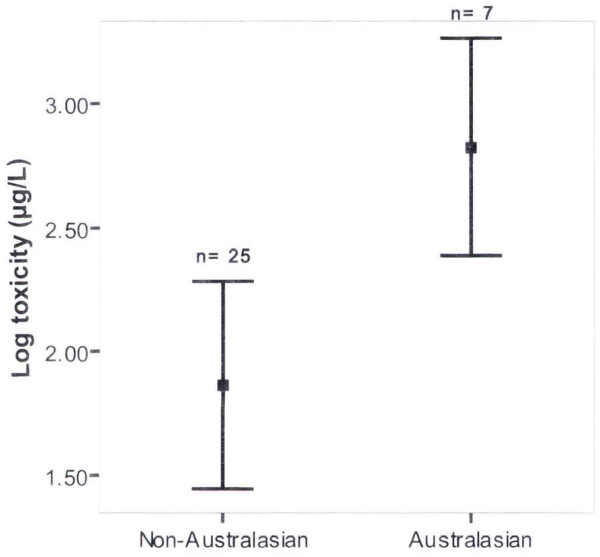

Figure 4.31 Log toxicity of copper to *P. japonicus* and *P. merguensis*.

4.3.12.5 Log toxicity of zinc to Crustacea in marine/estuarine water

A summary of the statistics for the log toxicity of Zn to marine/estuarine Crustacea is presented in Figure 4.32. The mean log toxicity and 95% confidence intervals of Zn to these Australasian and non-Australasian species are 3.10 (2.93 - 3.27) and 2.76 (2.49 - 3.03) respectively. A significant difference (t = -2.16, df = 73.8, p = 0.034, mod t-test) was detected between the two sets of organisms using a Student's t-test with the non-Australasian Crustacea being approximately twice more sensitive to Zn.

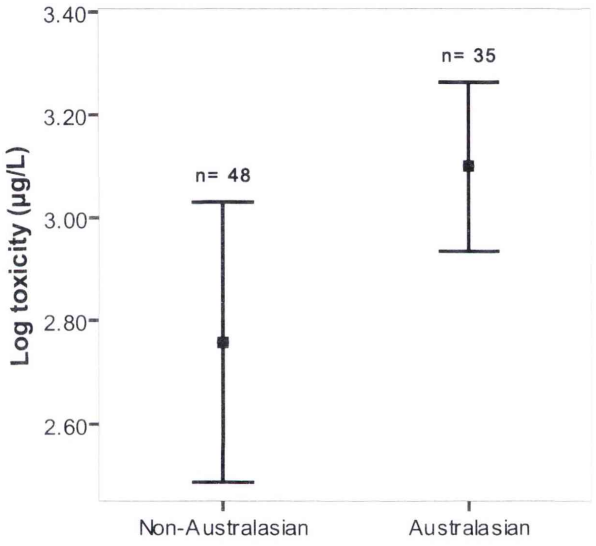

Figure 4.32 Log toxicity of zinc to non-Australasian and Australasian Crustacea.

4.3.13 Comparisons of marine/estuarine taxa – LC50

4.3.13.1 Log toxicity (LC_{50}) of cadmium to Crustacea in marine/estuarine water

A summary of the statistics for the log toxicity of Cd to marine/estuarine Crustacea is presented in Figure 4.33. The mean log toxicity and 95% confidence intervals of Cd to these Australasian and non-Australasian species are 3.25 (3.00 - 3.50) and 2.56 (2.33 - 2.80) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian Crustacea being five times more sensitive.

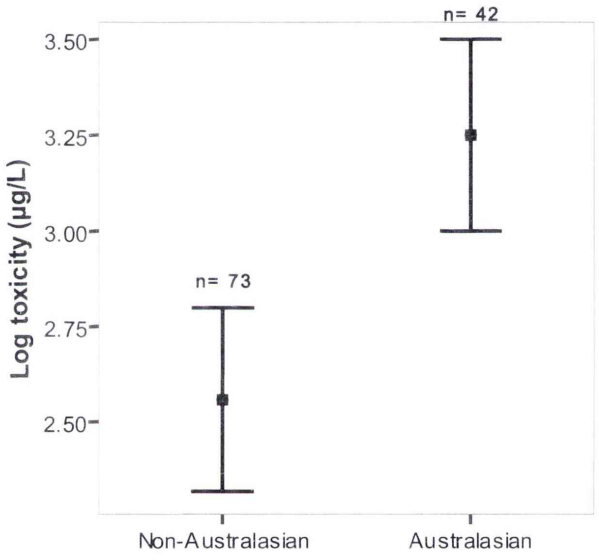

Figure 4.33 Log toxicity of cadmium to non-Australasian and Australasian Crustacea.

4.3.13.2 Log toxicity (LC_{50}) of copper to Chordata in marine/estuarine water

A summary of the statistics for the log toxicity of Cu to marine/estuarine Chordata is presented in Figure 4.34. The mean log toxicity and 95% confidence intervals of Cu to these Australasian and non-Australasian species are 3.32 (2.91 - 3.74) and 1.43 (0.39 - 2.48) respectively. As the 95% confidence intervals do not overlap, the sensitivity of the Australasian and non-Australasian organisms are significantly different with the non-Australasian Chordata approximately 80 times more sensitive to copper.

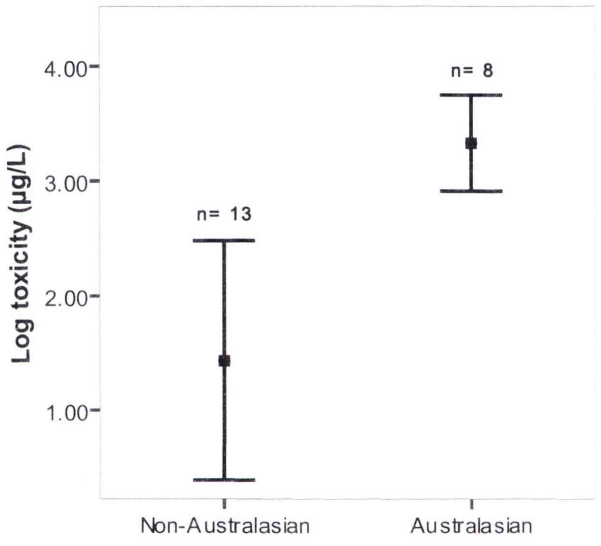

Figure 4.34 Log toxicity of copper to non-Australasian and Australasian Chordata.

4.3.13.3 Log toxicity (LC_{50}) of nickel to Crustacea in marine/estuarine water

A summary of the statistics for the log toxicity of Ni to marine/estuarine Crustacea is presented in Figure 4.35. The mean log toxicity and 95% confidence intervals of Ni to these Australasian and non-Australasian species are 3.80 (3.42 - 4.18) and 3.12 (2.43 - 3.82) respectively. A significant difference (t = -2.185, df = 15, p = 0.045) was detected between the two sets of organisms using a Student's t-test with the non-Australasian Crustacea being approximately five times more sensitive.

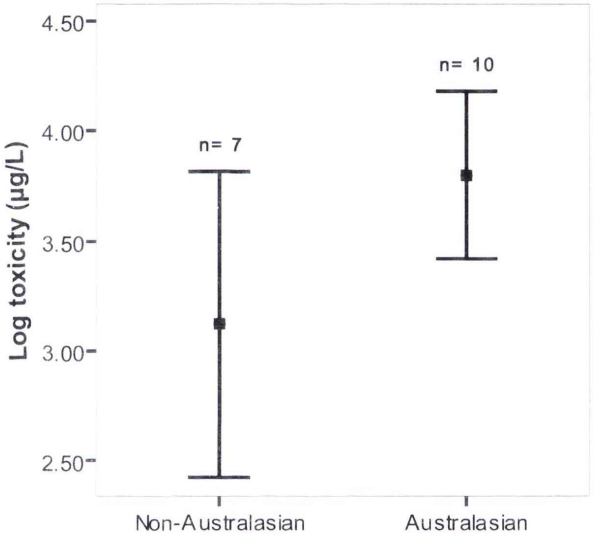

Figure 4.35 Log toxicity of nickel to non-Australasian and Australasian Crustacea.

4.3.13.4 Log toxicity (LC_{50}) of zinc to Crustacea in marine/estuarine water

A summary of the statistics for the log toxicity of Zn to marine/estuarine Crustacea is presented in Figure 4.36. The mean log toxicity and 95% confidence intervals of Zn to these Australasian and non-Australasian species are 3.15 (3.00 - 3.31) and 2.76 (2.49 - 3.03) respectively. A significant difference (t = -2.546, df = 71.1, p = 0.013, mod t-test) was detected between the two sets of organisms using a modified Student's t-test with the non-Australasian Crustacea being approximately three times more sensitive.

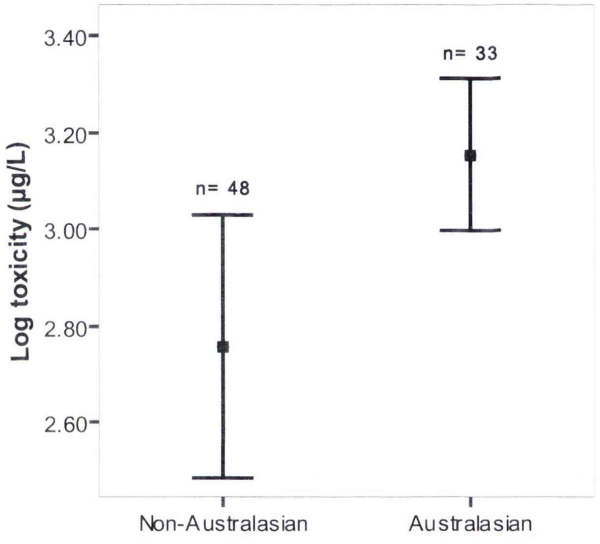

Figure 4.36 Log toxicity of zinc to non-Australasian and Australasian Crustacea.

4.4 SUMMARY OF 95% CONFIDENCE INTERVAL AND STUDENT T-TEST RESULTS

Due to the length of the above results section, the results of the statistical comparisons have been summarised into Table 4.10 for the freshwater comparisons and Table 4.11 for the marine/estuarine comparisons. These tables should greatly assist the reader as a quick reference for the following discussion of the results of the Student t-test and 95% confidence interval test section.

Table 4.10 Summary of the results of the comparisons of the sensitivity of Australasian and non-Australasian taxa in freshwater.

Metal/metalloid	Taxa	Toxicity data used			
		EC/LC_{50}	LC_{50}	EC_{50}	HM
As (V)	Chordata	NSD[1]	NSD	-[2]	-
	Chlorophyta	-	-	NSD	-
	Uniramia	Non-A[3]	Non-A	-	-
Cd	Chlorophyta	NSD	-	-	-
	Chordata	NSD	NSD	-	-
	Crustacea	NSD	NSD	NSD	-
	Magnoliophyta	NSD	-	-	-
	Mollusca	NSD	-	-	-
	Uniramia	NSD	NSD	-	-
Cr (VI)	Chlorophyta	NSD	-	Aust[4]	-
	Crustacea	NSD	-	NSD	-
	D. magna & *C.* cf. *Dubia*	NSD	-	-	-
	D. magna & *D. magna*	NSD	-	-	-
	D. pulex & *C.* cf. *dubia*	NSD	-	-	-
	D. pulex & *D. magna*	NSD	-	-	-
Cu	Chlorophyta	Aust	-	Aust	NSD
	Chordata	NSD	NSD	-	NSD
	Cnidaria	Aust	-	-	-
	Crustacea	NSD	NSD	Aust	Aust
	Mollusca	Non-A	Non-A	-	NSD
	C. robustus & *C. destructor*	NSD	-	-	Non-A
	C. dubia & *C.* cf. *Dubia*	NSD	-	-	NSD
	D. pulex & *C.* cf. *dubia*	Aust	-	-	Aust
	D. magna & *C.* cf. *Dubia*	NSD	-	-	NSD
	M. rosenbergi & *P. australiensis*	NSD	-	-	Aust
	P. subcapitata & *Chlorella sp.*	NSD	-	-	-
	S. quadricauda & *P. subcapitata*	Aust	-	-	-
	S. quadricauda & *Chlorella* sp.	Aust	-	-	-
	P. promelas & *M. splendida inornata*	Non-A	-	-	Non-A
	P. subcapitata (Non-A) & *P. subcapitata* (Aust)	NSD	-	-	-
	V. iris & *H. depressa*	NSD	-	-	NSD
Pb	Chordata	NSD	NSD	-	NSD
Hg	Chordata	Aust	Aust	-	-
	Crustacea	NSD	NSD	-	-
U	Chordata	Aust	-	-	-
	Crustacea	Aust	Aust	-	-
	D. magna & *M. macleayi*	Aust	-	-	-
Zn	Chlorophyta	NSD	-	NSD	-
	Chordata	NSD	NSD	-	Aust
	Crustacea	NSD	NSD	-	NSD
	D.. magna & *C.* cf. *Dubia*	NSD	-	-	NSD

[1] NSD = No Significant Difference ($p > 0.05$); [2] - = no statistical comparison could be made because one or both datasets contained insufficient or no data; [3]Non-A = non-Australasian taxa more sensitive; [4] Aust = Australasian taxa more sensitive.

Table 4.11 Summary of the results of the comparisons of the sensitivity of Australasian and non-Australasian taxa in marine/estuarine water.

Metal	Taxa	Toxicity Data Used		
		EC/ LC_{50}	LC_{50}	EC_{50}
Cd	Chordata	Non-A[1]	Non-A	-[2]
	Crustacea	Non-A	Non-A	-
	Echinodermata	NSD[3]	-	NSD
	Mollusca	NSD	-	-
Cu	Chordata	Non-A	Non-A	-
	Crustacea	NSD	NSD	-
	Echinodermata	Aust[4]	-	Aust
	Mollusca	NSD	NSD	NSD
	P. japonicus & *P. merguensis*	Non-A	-	-
Pb	Crustacea	NSD	NSD	-
Ni	Crustacea	NSD	Non-A	-
Z	Crustacea	NSD	Non-A	-
	Mollusca	NSD	NSD	NSD

[1]Non-A = non-Australasian taxa more sensitive; [2] - = no statistical comparison could be made because one or both datasets contained insufficient or no data; [3] NSD = No Significant Difference ($p > 0.05$); [4] Aust = Australasian taxa more sensitive.

4.5 Discussion

The combinations that are of most interest are either significantly different when EC/LC_{50} data is used but after being broken down into LC_{50}, EC_{50} or hardness modified data show no significant difference, or those that show no significant difference using the LC/EC_{50} data but become significantly different after being broken down into LC_{50}, EC_{50} or hardness modified data. This indicates the importance of data choice when doing these comparisons and also the importance of considering physicochemical factors such as water hardness.

4.5.1 Freshwater Comparisons

A total of 37 comparisons were made between Australasian and non-Australasian organisms for at least one of the four sets of toxicity data (i.e. a combination of EC and LC_{50} data, LC_{50}, EC_{50}, and hardness modified data) presented in Table 4.10. Of the 37 comparisons, 57% of these were not significantly different. Therefore 43% of the comparisons had at least one of the toxicity datasets showing significant differences between the Australasian and non-Australasian organisms. Of the data sets that were significantly different, Australasian taxa were found to be more sensitive in 84% of the cases and the non-Australasian taxa more sensitive in 16% of the cases.

There were a total of 70 possible comparisons made involving LC_{50} and EC_{50} data combined, LC_{50} data alone, EC_{50} data alone and hardness modified LC_{50} and EC_{50} combined data. Of the 70 comparisons, 63% of these showed no significant differences and 37% showed significant differences. These percentages are comparable to those reported in the previous paragraph. Of the data sets that were significantly different, Australasian species were more sensitive in 73% of the cases, while non-Australasian species were more sensitive in 27% of the cases.

There are a number of inherent problems with the above type of analysis. These problems are addressed individually below.

When comparing the sensitivity of different datasets, the same type of toxicity data should ideally be used. For example, both datasets should be 96 hour LC_{50} data. However, in the vast majority of cases there are insufficient data to permit such specific comparisons to be made. So in order to be able to make comparisons, less specific toxicity data were used. This does have implications: the more diverse the data, the greater the spread of values and, hence, the greater the variability and the lower the probability that statistically significant differences will be found. In other words, using less specific toxicity data makes it more difficult to obtain significant differences (if indeed there is) between the true sensitivity of Australasian and non-Australasian species.

This is definitely the case when LC_{50} and EC_{50} data are combined and used in comparisons. Given the above, a finding that an EC/LC_{50} comparison was not significantly different does not mean that a similar comparison made using more specific toxicity data (i.e. comparing only LC_{50} or only EC_{50} data) will necessarily also not be significantly different. Examination of the results in Table 4.10 indicates two cases (out of a possible 13) where a no significant difference result was obtained using EC/LC_{50} data which then become significantly different when EC50 data were used (i.e. copper and Crustacea, chromium (VI) and Chlorophyta).

Conversely, if an EC/LC_{50} data comparison is significantly different, then it is likely that similar comparisons with more specific toxicity data would also be significantly different. This occurred in all four cases (Table 4.10) where the EC/LC_{50} data were

significantly different and there were sufficient data to do more specific analysis. So in interpreting the results of Table 4.10, the results of comparisons using EC/LC_{50} data should only be used when similar comparisons could not be made using more specific data.

Another issue is that for some metals (i.e. Cd, Cr (III), Cu, Pb, Ni, Zn) it is known that water hardness affects their toxicity. If a comparison is being made using toxicity data for one of the above metals and the water used in the toxicity tests had a wide range of water hardness, then this may increase the variability of the toxicity data and make it less likely that significant differences will be detected. If, however, there was non-random variation in water hardness (e.g. the Australasian data had lower water hardness and the non-Australasian data had higher water hardness) then comparisons based on non-hardness modified toxicity data would increase the chances that a significant difference will occur. For the hardness modified metals, the only completely reliable comparisons are those made using hardness modified toxicity data. Once these two problems with the data presented in Tables 4.3 to 4.6 are taken into consideration, then the most correct/valid results of the available freshwater comparisons are shown in Table 4.12. With these problems taken into account, out of 37 possible comparisons approximately 57% showed no significant difference, whilst of the approximately 43% that had significant differences, the Australasian taxa were more sensitive in 80% of the cases and the non-Australasian taxa were more sensitive in 20% of the cases.

As well as just looking at the relative percentage of comparisons that are, and are not, significantly different, the relative toxicity can be examined quantitatively by calculating the ratio of the sensitivity of the non-Australasian and Australasian taxa. In calculating this ratio, the sensitivity of the Australasian taxa was always set to unity (Table 4.13). The mean of these ratios was calculated for all comparisons and for all comparisons where the ratio was not 1:1 (Table 4.13). A one-sided t-test (alternative hypothesis: mean does not equal one) was used to determine if the mean ratios were significantly different to one (i.e. one being the ratio if there is no difference in the sensitivity of the non-Australasian and Australasian taxa). If the mean ratios are significantly different to one then depending on the ratio, the non-Australasian organisms are significantly more or less sensitive than the Australasian species.

When the mean of all the ratios for the non-Australasian and Australasian species (i.e. 1.79, Table 4.13) was compared to a value of one, the ratio was found to be significantly larger ($p = 0.004$). Not surprisingly given the above result, when the mean ratio based on only the non 1:1 ratios (Table 4.13) was compared to one it was still significantly larger than one (i.e. 3.26). The probability of making a type II error (i.e. stating there was a difference when there was in fact no difference) was approximately halved from 4% to 2% (Table 4.13) when the 1:1 ratios were removed. Thus, overall the Australasian species were significantly more sensitive than the non-Australasian species.

Table 4.12 The results of the most reliable comparisons of the relative sensitivity of Australasian and non-Australasian freshwater taxa for each combination of metal and taxa.

Metal/metalloid	Taxa	Toxicity data used			
		EC/LC_{50}	LC_{50}	EC_{50}	HM
As (V)	Chordata		NSD[1]		
	Chlorophyta			NSD	
	Uniramia		Non-A[2]		
Cd	Chlorophyta	NSD			
	Chordata		NSD		
	Crustacea		NSD	NSD	
	Magnoliophyta	NSD			
	Mollusca	NSD			
	Uniramia		NSD		
Cr (VI)	Chlorophyta			Aust[3]	
	Crustacea			NSD	
Cu	Chlorophyta				NSD
	Chordata				NSD
	Crustacea				Aust
	Mollusca				NSD
	C. robustus & *C. destructor*				Non-A
	C. dubia & *C.* cf. *dubia*				NSD
	D. pulex & *C.* cf. *dubia*				Aust
	D. magna & *C.* cf. *dubia*				NSD
	M. rosenbergi & *P. australiensis*				Aust
	S. quadricauda & *P. subcapitata*	Aust			
	S. quadricauda & *Chlorella* sp.	Aust			
	P. promelas & *M. splendida inornata*				Non-A
	P. subcapitata (Non-A) & *P. subcapitata* (Aust)	NSD			
	V. iris & *H. depressa*				NSD
Pb	Chordata				NSD
Hg	Chordata		Aust		
	Crustacea		NSD		
U	Chordata	Aust			
	Crustacea		Aust		
	D. magna & *M. macleayi*	Aust			
Zn	Chlorophyta			NSD	
	Chordata				Aust
	Crustacea				NSD
	D. magna & *C.* cf. *dubia*				NSD

[1] NSD = No Significant Difference ($p > 0.05$); [2] Non-A = non-Australasian taxa more sensitive; [3] Aust = Australasian taxa more sensitive

Table 4.13 Ratio of the sensitivity of Non-Australasian and Australasian freshwater organisms for the most reliable comparisons.

Metal/ Metalloid	Taxa	Toxicity data used			
		EC/LC_{50}	LC_{50}	EC_{50}	HM
As (V)	Chordata		1:1		
	Chlorophyta			1:1	
	Uniramia		0.41:1		
Cd	Chlorophyta	1:1			
	Chordata		1:1		
	Crustacea		1:1	1:1	
	Magnoliophyta	1:1			
	Mollusca	1:1			
	Uniramia		1:1		
Cr (VI)	Chlorophyta			4.8:1	
	Crustacea			1:1	
Cu	Chlorophyta				1:1
	Chordata				1:1
	Crustacea				1.5:1
	Mollusca				1:1
	C. robustus & *C. destructor*				0.32:1
	C. dubia & *C.* cf. *Dubia*				1:1
	D. pulex & *C.* cf. *dubia*				2.6:1
	D. magna & *C.* cf. *dubia*				1:1
	M. rosenbergi & *P. Australiensis*				1.5:1
	S. quadricauda & *P. Subcapitata*	3.0:1			
	S. quadricauda & *Chlorella sp.*	5.3:1			
	P. promelas & *M. splendida inornata*				0.28:1
	P. subcapitata (Non-A) & *P. subcapitata* (Aust)	1:1			
	V. iris & *H. depressa*				1:1
Pb	Chordata				1:1
Hg	Chordata		3.7:1		
	Crustacea		1:1		
U	Chordata	3.4:1			
	Crustacea		5.5:1		
	D. magna & *M. macleayi*	6.9:1			
Zn	Chlorophyta			1:1	
	Chordata				7.1:1
	Crustacea				1:1
	D. magna & *C.* cf. *dubia*				1:1
Data used to calculate the mean ratio	**Mean ratio and 95% CIs (for all data types)**	**Mean ratio and 95% CIs for LC/EC_{50} data**	**Mean ratio and 95% CIs for LC_{50} data**	**Mean ratio and 95% CIs for EC_{50} data**	**Mean ratio and 95% CIs for HM data**
For all comparisons	1.79 (1.27 – 2.31)	2.08 (1.14 – 3.02)	1.83 (0.6 – 3.07)	1.76 (-0.37 – 3.89)	1.49 (0.66 – 2.32)
Prob. of mean ratio being sig. diff. to 1 for all comparisons	0.004	0.041	0.232	0.374	0.270
For all non 1:1 comparisons	3.26 (2.11 – 4.41)	4.24 (2.65 – 5.83)	3.21 (0.29 – 6.13)	nc	2.22 (0.18 – 4.26)
Prob of mean ratio being sig. diff. to 1 for all non 1:1 comparisons	0.002	0.016	0.277	nc	0.294

When the non-Australasian and Australasian groups are separated into the different kinds of data used (e.g. EC/LC_{50}, LC_{50}, EC_{50} and HM), the effect that the type of data

had on the differences between the mean sensitivities can be investigated. Of the four groups of toxicity data, only the mean ratio of the EC/LC_{50} data (based on all the ratios) were significantly different to the value of one ($p = 0.041$) with the Australasian species being significantly more sensitive than the non-Australasian taxa. This indicates that when the data are broken down into more specific groups the differences are not significant. This may be due to a number of factors, with the most likely of these being a reduction in sample size leading to decreased degrees of freedom in the statistical analysis.

4.5.2 Marine/estuarine water comparisons

Table 4.11 details the comparisons that were undertaken on each metal/metalloid and taxa combination for marine/estuarine organisms. There were a total of 13 combinations involving EC/LC_{50} data, nine involving LC_{50} data only and four involving EC_{50} data only.

There were six combinations of data that were significantly different for at least one of the sets of toxicity data (i.e. EC/LC_{50}, LC_{50} only or EC_{50} only). As indicated in section 4.4.1 the most reliable comparisons occur when the same type of toxicity data are used (e.g. LC_{50} vs LC_{50} data). Where comparisons could be made using the LC_{50} only data and the EC_{50} only data they yielded the same result as those from the EC/LC_{50} comparisons, except for the nickel and zinc crustacean comparisons (Table 4.14). In these two cases, the EC/LC_{50} comparison indicated there was no significant difference while the LC_{50} comparisons indicated that, in both cases, the non-Australasian species were significantly more sensitive than the Australasian species. By removing the combined EC and LC_{50} data, the most reliable results available from the analysis are presented in Table 4.14.

Table 4.14 The results of the most reliable comparisons of the relative sensitivity of Australasian and non-Australasian marine/estuarine species for each combination of metal and taxa.

Metal	Taxa	Toxicity Data Used		
		EC/LC_{50}	LC_{50}	EC_{50}
Cd	Chordata		NSD[1]	
	Crustacea		Non-A[2]	
	Echinodermata			NSD
	Mollusca	NSD		
Cu	Chordata		Non-A	
	Crustacea		NSD	
	Echinodermata			Aust[3]
	Mollusca		NSD	NSD
	P. japonicus & *P. merguensis*	Non-A		
Pb	Crustacea		NSD	
Ni	Crustacea		Non-A	
Zn	Crustacea		Non-A	
	Mollusca		NSD	NSD

[1] NSD = No Significant Difference ($p > 0.05$); [2] Non-A = non-Australasian taxa more sensitive; [3] Aust = Australasian taxa more sensitive

There is a total of 15 reliable comparisons of the sensitivity of Australasian and non-Australasian marine/estuarine organisms. Of these, approximately 40% (i.e. 6 out of 15) were significantly different. Where there were significant differences in sensitivity the non-Australasian organisms were more sensitive in 86% of the cases (i.e. five out of six occasions).

When only the LC_{50} data were compared, 45% of the comparisons (four out of nine) where significantly different, and in all of these cases the non-Australasian organisms were more sensitive than the Australasian organisms. For the four EC_{50} comparisons 25% (one out of four) was significantly different, and in this case the Australasian organisms were more sensitive. Table 4.15 presents, for the most reliable comparisons, the ratios of the non-Australasian to Australasian marine/estuarine taxa with the value of the Australasian species always being one. The same statistical tests were conducted on this dataset as was done for the freshwater toxicity dataset (Section 4.4.1).

Table 4.15 Ratio of sensitivity of Non-Australasian and Australasian marine/estuarine organisms for the most reliable comparisons.

Metal	Taxa	Toxicity Data Used		
		EC/LC_{50}	LC_{50}	EC_{50}
Cd	Chordata		1:1	
	Crustacea		0.21:1	
	Echinodermata			1:1
	Mollusca	1:1		
Cu	Chordata		0.013:1	
	Crustacea		1:1	
	Echinodermata			2:1
	Mollusca		1:1	1:1
	P. japonicus & *P. merguensis*	0.11:1		
Pb	Crustacea		1:1	
Ni	Crustacea		0.21:1	
Zn	Crustacea		0.4:1	
	Mollusca		1:1	1:1
Data used to calculate the mean ratio	Mean ratio and 95% CIs (for all data types)	Mean ratio and 95% CIs for LC/EC_{50} data	Mean ratio and 95% CIs for LC_{50} data	Mean ratio and 95% CIs for EC_{50} data
For all comparisons	0.74 (0.46 – 1.02)	0.56 (nc[1])	0.55 (0.26 – 0.84)	1.25 (0.76 – 1.74)
Prob. of mean ratio being sig. diff. to 1 for all comparisons	0.081	0.500	0.015	0.391
For all non 1:1 comparisons	0.44 (-0.08 – 0.96)	nc	0.19 (0.06 – 0.32)	nc
Prob of mean ratio being sig. diff. to 1 for all non 1:1 comparisons	0.077	nc	0.000	nc

[1] nc – not calculable as there were too few data.

When, as in the freshwater section, the mean ratios of the sensitivities of the non-Australasian and Australasian organisms were compared to one, no significant differences were detected, for the mean ratios calculated both with and without the 1:1 ratios. Thus overall, there is no significant difference in the sensitivity of marine/estuarine species from Australasia and non-Australasia.

Of the data groups (EC/LC_{50}, LC_{50}, EC_{50}) the LC_{50} data indicated that the mean sensitivities of the non-Australasian organisms were significantly ($p = 0.015$) more sensitive than the Australasian organisms. This significance increased ($p = 0.0002$) when the 1:1 ratios were removed from the LC_{50} analysis.

4.5.3 Bonferroni Adjustment

Customarily the alpha level (the probability at which significant differences are inferred) is set at 0.05, which means on average only one in twenty statistical tests will determine a significant difference when in fact there is no difference (i.e. a type I error). When multiple statistical tests are conducted within a study, the chance of making at least one type I error increases in accordance with the formula:

Probability of making at least one type I error = $1 - (1 - \alpha)^k$;

where α is the level of significance used for each test (typically 0.05) and k is the number of tests repeated in the study.

Therefore as the number of repeat statistical tests increases, the probability of making a type I error also increases. For example, in five tests the probability of finding at least one significant difference due to chance equals 0.2, or one in five, while in ten tests this probability increases to 0.40, which is approaching one in two (Sankoh *et al.* 1997).

To overcome this problem, a number of statistical tests have been developed. The most widely used of these is the Bonferroni adjustment. In this method the alpha value is divided by the number of repeat experiments or statistical comparisons being made. For example if α equals 0.05 and there are five statistical tests then the new probability at which significance is inferred would be 0.01. The Bonferroni adjustment however, is overly conservative (i.e. it makes it unnecessarily hard to find significant differences).

In studies such as this, one can use the results without adjustments, knowing that a certain number of results which were found to be significantly different will be invalid (i.e. type I errors). Alternatively one can use a more conservative method such as the Bonferroni adjustment to reduce the chance of making type I errors. The problem with this approach is that the probability of making type II errors increases (i.e. concluding there is no difference when in fact there is a difference).

In this study, thirty seven comparisons were made for the freshwater organisms (Tables 4.5 and 4.6). This means that with an alpha level of 0.05, approximately 2 of the 15 results found to be significantly different would be incorrect. However, not all of these 37 comparisons were made using the t-test method with an alpha of 0.05. Of the 37 comparisons made, 22 were determined by t-tests and 15 were determined using the 95% confidence interval method. None of the t-test based comparisons resulted in differences in the sensitivity of the Australasian species, so these results should all be correct. For the comparisons made using the 95% confidence intervals with the degrees of freedom typically found in this study, the alpha value is approximately 0.01 (Ray Correll *pers. comm.*) rather than the usual 0.05. This means that on average only one test in 100 will make a type I error using the 95% confidence interval method. Given that 15 comparisons were made by this method it is highly likely that all of the significant differences reported by this method are correct (i.e. no type I errors). Therefore, overall the probability that any of the comparisons that were found to be significantly different are incorrect is very low.

A similar analysis was used for the marine/estuarine results, where only 15 comparisons were made, indicating that with an alpha of 0.05 less than one of the significant results would be incorrect. Again the probability of type I errors occurring is very low. Because the probability of type I errors for the freshwater and marine/estuarine analyses is low, it was felt that there was no need, and in fact it would be counter-productive to use the Bonferroni adjustment.

The statistical comparison of the mean ratios to a value of one was conducted so few times that the probability of type I errors was low and therefore the Bonferroni adjustment was not applied to these results.

4.5.4 Power analysis

A post hoc power analysis was undertaken on those comparisons that were found to be not significantly different ($p > 0.05$) and the results are presented along with the other test statistics in Tables 4.3 – 4.9. Power analysis provides an indication of the ability of the statistical test to detect significant differences given the data that is available. Values

below 0.80 indicate that there is a greater probability that a significant difference may be present but it is not detected. It can be seen that the power is typically low for most of these comparisons. This is not unexpected due to the low number of samples used for some of these comparisons. This finding re-iterates the general paucity of toxicity data for species both in Australasia and outside this region.

4.5.5 Comparison with previous studies

The comparison of sensitivity of Australasian and non-Australasian organisms to a variety of toxicants has previously been investigated, and a summary of the outcomes of each study is provided in Table 4.16. The results of these studies indicated that there was no overall pattern to the relative sensitivity of Australasian and non-Australasian organisms to a variety of chemicals, including organics, pesticides, polar narcotics, metals (Cu and U) and non-polar narcotics.

Table 4.16 Summary of the results of other studies comparing the relative sensitivities of Australasian and Non-Australasian species.

Study	Chemicals	Sensitivity
Johnston *et al.* (1990)	Organics	Aust ≤ Non-Aust
Sunderam *et al.* (1992)	Pesticides	Aust < Non-Aust
Davies *et al.* (1994)	Pesticides	Aust > Non-Aust
Mulhall (1997)	Polar narcotics	Aust < Non-Aust
Markich and Camilleri (1997)	Cu and U	Aust = Non-Aust
Rose *et al.* (1998)	Non-polar narcotics	Aust > Non-Aust
Westbury *et al.* (2004)	Polar narcotics	Aust < Non-Aust
Phyu (2004)	Atrazine and Molinate	Aust = Non-Aust
Hose and Van den Brink (2004)	Endosulfan	Aust = Non-Aust

Direct comparisons of the results of these literature studies and the analyses conducted in this chapter are not possible due to the methods that were used to compare the groups or the compounds were different to those investigated here. The one exception being the study conducted by Markich and Camilleri (1997). The authors found that there were no significant differences in the acute toxicity of copper and uranium to the Australian tropical freshwater fish, the purple spotted gudgeon (*Mogurnda mogurnda*) and the temperate freshwater fish, the common jollytail (*Galaxias maculatus*). The authors concluded that the Australian water quality guidelines for copper, derived largely from North American toxicity data, were appropriate for Australian conditions when key water quality variables (e.g. temperature, water hardness and alkalinity) were considered. A similar finding was also found in the current study, with the non-

Australasian and Australasian freshwater fish species showing no significant differences in sensitivity when exposed to copper after the adjustment of water hardness (see the chordate comparison for Cu in Table 4.13). Due to the much greater number of species used in the current study for the comparison of the sensitivity of Australasian and non-Australasian chordates to copper (15 Australasian species and 22 non-Australasian species), the confidence of the finding that non-Australasian toxicity data for Chordata would adequately protect Australasian Chordata has increased.

The current study found that Australasian freshwater fish were significantly more sensitive to uranium than non-Australasian fish (see the chordata comparison for U in Table 4.13) whilst Markich and Camilleri (1997) found there was no significant difference between the two groups. It should be noted that the number of species for which there were uranium toxicity data available, for both the current and the Markich and Camilleri (1997) study, was limited - with 80% of the data in both cases being made up of Australasian species. With the greater number of species involved in the current study (five additional Australasian and nine additional non-Australasian species) greater confidence can be placed in the result that there is a difference in the sensitivities of Australasian and non-Australasian Chordata to uranium.

Maltby *et al.* (2003), Leung *et al.* (2003), Kwok *et al.* (2007) and Chapman *et al.* (2006) investigated the relative sensitivities of organisms from different regions to a range of different chemicals. Maltby *et al.* (2003) found that there were no significant differences ($p \leq 0.05$) between European and North American arthropods for three insecticides (i.e. chlorpyrifos, diazinon and fenitrothion), and that it was relatively safe to use North American ecotoxicity data to derive water quality guidelines for European ecosystems, and vice versa, for a selected range of insecticides. Leung *et al.* (2003), who compared a much larger set of chemicals (metals, organics and in-organics) than Maltby *et al.* (2003), concluded that using temperate species data to develop water quality guidelines for tropical or subtropical regions could be done safely using an extrapolation factor of 40. Additional data and further analysis of the work by Leung *et al.* (2003), by Kwok *et al.* (2007), found that the tropical species were both more and less sensitive than the temperate species and they concluded that an extrapolation factor of 10 was suitable to permit WQGs derived from temperate toxicity data to protect tropical ecosystems. Chapman *et al.* (2006) found substantive differences between three geographic regions

(polar, temperate and tropical) but that there was no pattern of increased toxicity from polar to tropical regions and that sensitivity data from one region will not be universally protective of other regions.

The lack of significant differences between the three chemicals studied by Maltby *et al.* (2003) highlights the importance of comparing the sensitivity of species from different regions for a large number of chemicals. While the results for those chemicals are probably correct, the results from studies investigating a greater number of chemicals indicated that there is no clear pattern of relative sensitivity. Therefore, when the relative sensitivity of species to more chemicals is compared, the results of Maltby *et al.* (2003) may ultimately be found to be misleading. Caution should be practiced when extrapolating from so few comparisons of sensitivity and using those results for a wider range of insecticides.

This lack of a consistent pattern in the relative sensitivity of species from different regions found by Kwok *et al.* (2007) and Chapman (2006) was also found in the current study. This lack of a consistent pattern may be resolved when more chemicals are analysed.

Kwok *et al.* (2007) used a species sensitivity distribution method to determine that an extrapolation factor of 10 was required for chemicals that had not been tested using tropical species in order for temperate species data to generally be protective of tropical ecosystems. In a later chapter a similar approach will be applied to this study. But in this chapter mean ratios of the sensitivities of non-Australasian to Australasian species were determined. These values indicate that on average the freshwater Australasian species are 1.79 times more sensitive than non-Australasian species and the Australasian marine/estuarine species are 1.35 times less sensitive than the non-Australasian species (i.e. the EC/LC values for the Australasian species are on average 135% the size of those for non-Australasian species). The extrapolation factor determined by Kwok *et al.* (2007) is over 5 times greater than the difference found in the current study between Australasian and non-Australasian freshwater organisms and is 10 times greater than the difference found between Australasian and non-Australasian marine/estuarine organisms. The difference in the results of Kwok *et al.* (2007) and the current study may reflect that the ratios (extrapolation factors) were derived from different data.

The findings of the t-tests conducted in this chapter indicated that non-Australasian data are not sufficient to protect freshwater Australasian aquatic ecosystems from metal toxicity. Non-Australasian data, however, was found to adequately protect our local marine/estuarine ecosystems from metal toxicity. As there are numerous other factors involved in the protection of our ecosystems using toxicity data, we cannot guarantee that the inherent problem of using non-Australasian data to protect those ecosystems has been overcome.

4.6 CONCLUSIONS

- When using the most reliable data set available for each combination of the 37 freshwater comparisons, 43% were significantly different. Of those that were found to be significantly different, the Australasian taxa were more sensitive in 84% of the cases.
- Ratios of the sensitivity of all non-Australasian and Australasian taxa indicated that the Australasian species were significantly more sensitive than the non-Australasian species.
- When using the most reliable data set available for each combination of the 15 marine/estuarine comparisons, 40% were significantly different. Of those that were found to be significantly different, the non-Australasian taxa were more sensitive in 86% of the cases.
- When using ratios of the sensitivity of all non-Australasian and Australasian taxa it was found the non-Australasian marine/estuarine species were not significantly more sensitive than the corresponding Australasian species.
- The magnitude of the difference in the sensitivity of the Australasian species to the non-Australasian species for fresh and marine/estuarine water were 1.79 and 0.74 respectively.
- If non-Australasian species toxicity data were used to protect Australasian ecosystems, freshwater ecosystems would not be adequately protected but marine/estuarine ecosystems would be adequately protected.

5 COMPARISON OF THE SENSITIVITY OF AUSTRALASIAN AND NON-AUSTRALASIAN SPECIES TO SELECTED METALS USING SPECIES SENSITIVITY DISTRIBUTIONS

5.1 INTRODUCTION

The species sensitivity distribution (SSD) concept is frequently used in ecological risk assessments (e.g. Solomon *et al.* 1996, 2001; Giesy *et al.* 1999; Muschall & Warne 2003) and in deriving water quality guidelines (WQG) (i.e. Australia and New Zealand (ANZECC & ARMCANZ 2000), Denmark (Petersen & Pedersen 1995), South Africa (Roux *et al.* 1996), The Netherlands (Van de Plassche *et al.* 1993), and the Unites States of America (Stephan *et al.* 1985; USEPA 1986). The aim of SSD is to determine the concentration of a toxicant that is protective of a selected percentage of species (usually 95%) in the environment. Species sensitivity distributions are constructed by fitting a cumulative distribution function to a plot of species sensitivity (i.e. toxicity) data against rank assigned percentiles (Kooijman 1987, Van Straalen and Denneman 1989, Wheeler *et al.* 2002). From the cumulative distribution, the concentration that is protective of 95% of species (i.e. the PC95) or any other selected percentage of species can be calculated. While the usual level of protection is 95% in WQG, other levels have also been used. For example, the Australian and New Zealand WQG provide at least four different levels of protection – 99%, 95%, 90% and 80% of species (ANZECC & ARMCANZ 2000). A similar approach with multiple levels of protection has also been recommended for the freshwater WQG for toxicants in South Africa (Warne *et al.* 2004).

In Australasia, SSD curves and PC95 values have been used to derive WQG for toxicants, with an emphasis on using Australasian or site specific data where available (ANZECC and ARMCANZ 2000, Chapman *et al.* 2001). The utility of non-Australasian toxicity data to address local problems has been explored, particularly in Australia where the trans-hemisphere application of toxicity has long been questioned (Johnston *et al.* 1990, Sunderam *et al.* 1992, Davies *et al.* 1994). Australasian and non-Australasian toxicity data have only been compared once using the SSD approach. Hose and Van den Brink (2004) compared the sensitivity of fish, invertebrates and amphibians to the organochlorine insecticide, endosulfan, using SSD and also

investigated whether SSD derived from laboratory data would adequately protect organisms in the field. They found that there was no significant ($p > 0.05$) difference between the sensitivity of the Australasian and non-Australasian fish and arthropods (a group comprised of insects and crustaceans) and that laboratory data adequately protected organisms in the field.

Maltby *et al.* (2003) investigated the use of SSD in pesticide risk assessment and focussed on data and model selection for constructing SSD. The authors investigated the potential threshold concentrations derived from SSD to protect natural assemblages from the adverse effects of pesticides. An objective of the study was to use a single-species toxicity database, collated by the authors, to investigate how the species' identity and distribution model influenced the assessment of risk. The authors found that there were enough data to compare the response of freshwater and saltwater arthropods for six insecticides, flowing water and standing water arthropods for four insecticides, tropical and temperate arthropods for three insecticides, and European and North American arthropods for three insecticides. It was concluded that there was no statistically significant ($p > 0.05$) difference in the cumulative distribution function for arthropods from temperate and tropical regions, from Europe or North America, or from flowing water or standing water habitats. Therefore, the authors tentatively concluded that it would be relatively safe to use North American ecotoxicity data to derive WQG for European ecosystems and vice versa, for a selected range of insecticides.

Similarly, Kwok *et al.* (2007) compared the sensitivity of tropical and temperate freshwater species to nine metals, seven pesticides, phenol and ammonia and found that "for most metals, temperate species tend to be more sensitive than their tropical counterparts. However, for unionised ammonia, phenol and some pesticides (e.g. chlorpyrifos), tropical species are likely more sensitive". They concluded that using temperate species data to develop WQG for tropical or subtropical regions could be done safely using an extrapolation factor of 10 for chemicals never tested with tropical species.

In this chapter comparisons between Australasian and non-Australasian species sensitivity to metals were made using the database collated and discussed in earlier chapters. Due to the minimum data requirements of SSD methods and the amount and

type of toxicity data that was available comparisons could only be conducted for Cd, Cu and Zn.

5.2 METHODS

5.2.1 Selection of metals

The selection of metals for SSD analysis was based upon having sufficient toxicity data for both Australasian and non-Australasian species to meet the minimum data requirements of SSD. It is recommended by the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ 2000) and the OECD (1995) that a minimum of data for five species that belong to at least four taxonomic groups be used to generate SSD. The definition of taxonomically different organisms used in the Australian and New Zealand WQG are presented in Table 5.1. The metals that met this criterion are Cd, Cu and Zn.

Table 5.1 Types of taxonomically different organisms and the major subdivisions of organisms these belong to.

Major subdivisions of organisms	Type of organisms that are considered as being taxonomically different
Fish	fish
Invertebrates	crustaceans, insects, molluscs, annelids, echinoderms, rotifers, hydra
Plants	green alga, brown algae, red algae, macrophytes
Others	blue-green algae (cyanobacteria), amphibians, bacteria, protozoans, coral, fungi and others

Table modified from Warne 2001.

5.2.2 Toxicity data

Toxicity data for Cd, Cu and Zn were obtained from the Australasian Ecotoxicology database (Warne *et al.* 1998, Warne & Westbury 1999, Markich *et al.* 2002) and the non-Australasian Ecotoxicity database (Appendices 1 - 4). Only one toxicity datum is used to represent each species in the SSD methods. In the case of a species only having one toxicity value available, that value was used to represent the species. Where multiple data were available for only one endpoint for a particular taxon, the geometric mean of those values was used. If there were multiple data for multiple endpoints, the endpoint with the lowest geometric mean was taken to represent the species as per the rules used in the Australian and New Zealand WQGs (ANZECC & ARMCANZ 2000, Warne 2001).

Toxicity data were limited to acute LC50 and EC50 values from studies with defined exposure periods. Toxicity data were considered 'Australasian' if test organisms occur in natural ecosystems of Australasia and/or tests were conducted under local conditions (such as local river water). Under this definition, data for introduced species (in this case: European carp: *Cyprinus carpio,* mosquito fish: *Gambusia holbrooki* and rainbow trout: *Oncorhynchus mykiss*) could be included where tests were conducted in Australasia using local water and appropriate temperature conditions. Data from tests on these species conducted elsewhere were considered non-Australian. By classifying data as being Australasian or non-Australasian, a direct comparison between SSD curves obtained from Australasian species and non-Australasian species could be made.

5.2.3 Data analysis

Species sensitivity distributions (SSD) were fitted separately to the Australasian and non-Australasian data for each metal using the BurrliOZ program (Campbell *et al.* 2000). BurrliOz uses a maximum likelihood method to select the distribution from the Burr Type III family of distributions, that best fits the data (Shao 2000). The Burr Type III distributions are flexible three-parameter distributions. This family of distributions includes the log-logistic distribution (used in the Aldenberg and Slob (1993) method by the Dutch), and can provide good approximations of the log-normal distribution (used in the Wagner and Lokke (1991) method by the Danish), and the triangular distribution (used in the Stephan *et al.* (1985) method by the USA). As such, it should theoretically select a distribution that fits the data at least as well as each of the previously mentioned three SSD methods (Shao 2000).

From the fitted SSDs the PC95 and PC50 values were calculated. Strictly, these are the PC95 and PC50 values with 50% confidence (i.e. approximately 50% of the PC95 values will protect less than 95% of species and 50% will protect more than 95% of species). These are often denoted by the terms PC95 50% and PC50 50%, however for simplicity sake we will simplify this to PC95 and PC50 The BurrliOZ software calculates confidence intervals (CI) for PC values using a bootstrap technique (Campbell *et al.* 2000). As a result, CIs may vary with subsequent re-runs even though

the same toxicity data are used. We estimated 95% CIs for the PC95 and PC50 values by calculating the 2.5% and 97.5% confidence intervals. The 2.5 and 97.5% intervals were estimated 10 times with the geometric mean of these 10 estimations used as the lower and upper limits of the 95 % CI.

Non-overlapping 95% CIs were used as the criterion to determine significant differences between the PC95 and PC50 values for the Australasian and non-Australasian SSDs. However, while non-overlapping 95% CIs indicate that there are significant differences, when the 95% CIs overlap it cannot be inferred whether the means are significantly different or not-significantly different (Barr 1969; Nelson 1989; and Lo 1994). In such cases, one must use a statistical test to determine whether there are significant differences. In this study, we used the standard error of the difference test (Sprague and Fogels 1977) to determine if the PC95 and PC50 values for the Australasian and non-Australasian SSDs were statistically different.

In order to be able to compare the PC95 values from the Australasian and non-Australasian SSDs to the PC95 values of the Australian and New Zealand WQGs, an acute-to-chronic ratio (ACR) was used. Acute to chronic ratios are the ratio of the acute toxicity to the chronic toxicity data for a particular chemical and are calculated using the following formula:

ACR = acute toxicity/chronic toxicity

The acute and chronic data did not have to have the same measure of toxicity or endpoint but, they must be for the same species, and have been presented in the same paper or at least determined in the same laboratory.

The ACRs that were used in this study were those that were calculated to derive the Australian and New Zealand WQGs (ANZECC and ARMCANZ 2000) with the values derived from these ACRs denoted as estimated chronic values.

5.3 RESULTS

5.3.1 Comparison of Australasian and non-Australasian SSDs and PC values

5.3.1.1 Cadmium – freshwater

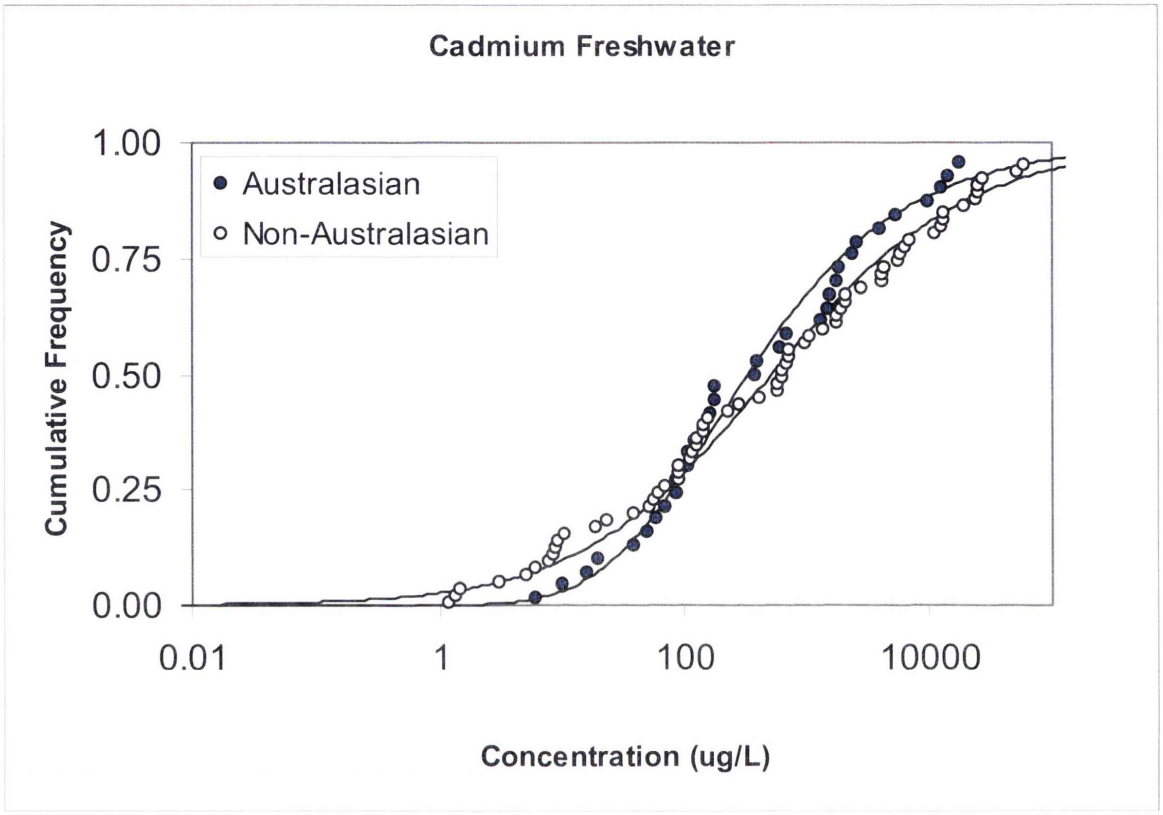

Figure 5.1 Species sensitivity distributions for Australasian and non-Australasian species acutely exposed to Cd in freshwater.

A total of 35 toxicity data points was used for the Australasian SSD for Cd in freshwater and 68 were used for the non-Australasian SSD (Table 5.1). The distributions have different gradients and therefore cross over each other at about 25% of the cumulative distribution. From cumulative frequencies between 0 and 25% the Non-Australasian SSD lies to the left of that for the Australasian, whilst from a cumulative frequency greater than 25% the distribution for the Australasian species is to the left. This indicates that the most sensitive non-Australasian species are more sensitive than the Australasian taxa. The 95% CIs for the Australasian and non-Australasian PC95 and PC50 values overlap (Table 5.2). Therefore the PC values were compared using the standard error of the difference test (Sprague & Fogels 1977) which indicated that there were no significant differences ($p > 0.05$) between the Australasian and non-Australasian PC95 values and between and the corresponding PC50 values.

Table 5.2 Protective Concentrations (PC) for Australasian and non-Australasian species exposed to Cd in freshwater (µg/L).

PC values	Australasian Acute	Non-Australasian Acute
95 (95% CI)	14.2 (5.6-37.6)	2.9 (0.73-9)
50 (95% CI)	338 (159-1095)	527 (185-1810)

5.3.1.2 Cadmium – marine/estuarine

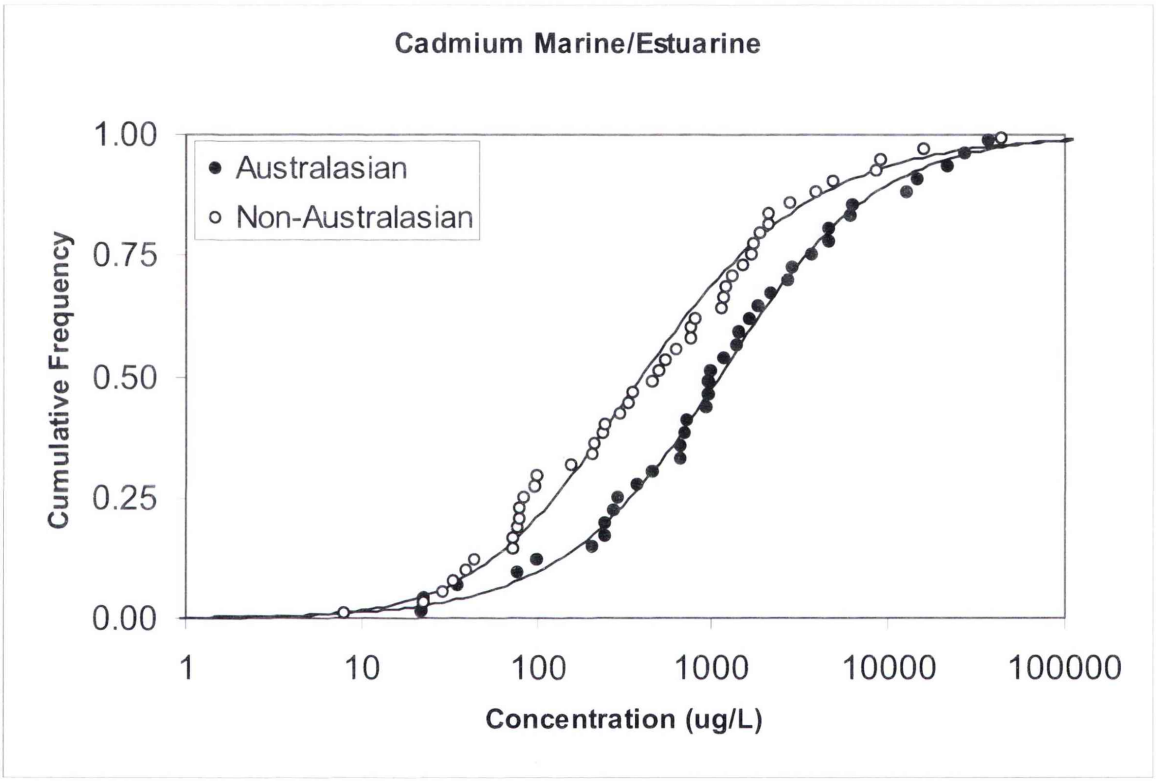

Figure 5.2 Species sensitivity distributions for Australasian and non-Australasian species acutely exposed to Cd in marine/estuarine water.

A total of 38 toxicity data points was used for the Australasian SSD for Cd in marine/estuarine water and 46 were used for the non-Australasian SSD (Figure 5.2). Except at both extremes of the cumulative distribution (i.e. cumulative frequencies of 0 and 100%) the SSD for the non-Australasian species is to the left of that for the Australasian species indicating that they may be more sensitive. The 95% CI for the Australasian and non-Australasian PC95 and PC50 values overlap (Table 5.3). Therefore the standard error of the difference test (Sprague & Fogels 1977) was used to compare the data. This indicated that there were no significant differences ($p > 0.05$) between the Australasian and non-Australasian PC95 and PC50 values.

Table 5.3 Protective Concentrations (PC) for Australasian and non-Australasian species exposed to Cd in marine/estuarine water (µg/L).

PC values	Australasian Acute	Non-Australasian Acute
95 (95% CI)	46.8 (16.2-165)	24.5 (10.3-52.3)
50 (95% CI)	1100 (619-2190)	397 (216-845)

5.3.1.3 Copper – freshwater

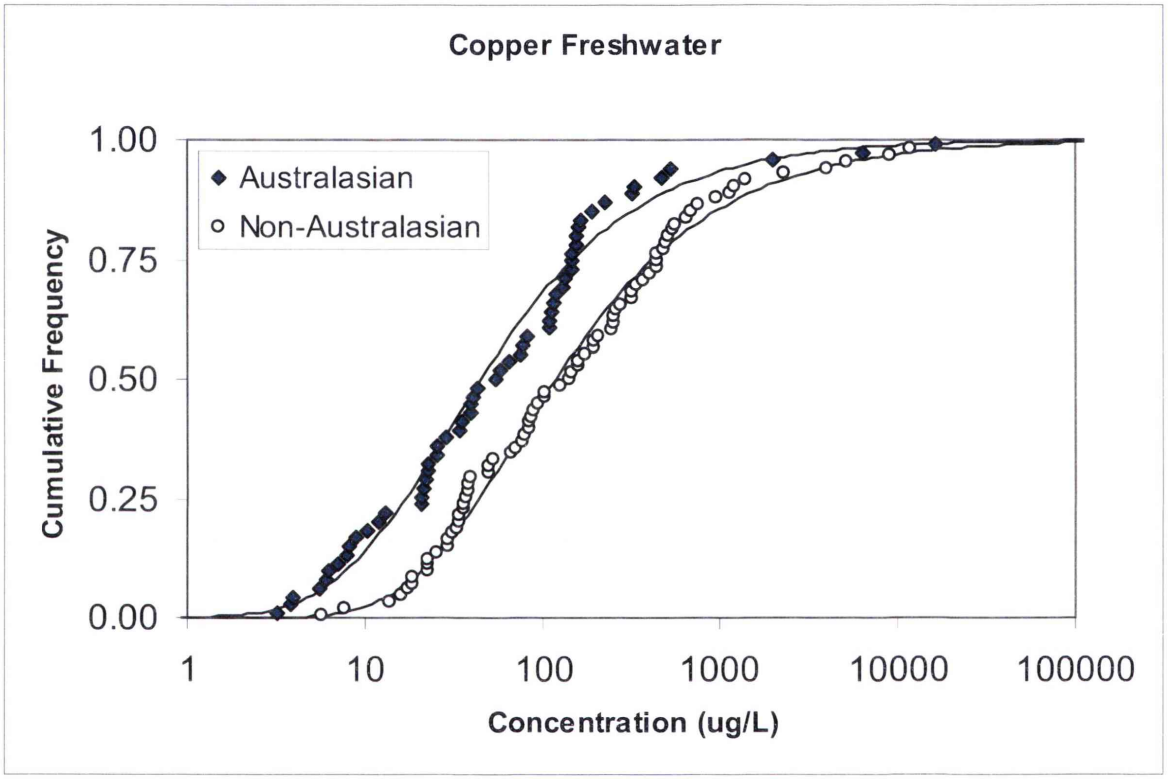

Figure 5.3 Species sensitivity distributions for Australasian and non-Australasian species acutely exposed to Cu in freshwater.

A total of 57 toxicity data points was used for the Australasian SSD for Cu in freshwater and 77 were used for the non-Australasian SSD (Figure 5.3). The SSD for the Australasian species lies to the left of that for the non-Australasian species indicating that they may be more sensitive. The 95% CIs for the Australasian and non-Australasian PC95 and PC50 values do not overlap (Table 5.4). Therefore the Australasian species are significantly more sensitive to copper than the non-Australasian species.

Table 5.4 Protective Concentrations (PC) for Australasian and non-Australasian species exposed to Cu in freshwater (µg/L).

PC values	Australasian Acute	Non-Australasian Acute
95 (95% CI)	5.2 (3.25-8.3)	14.2 (9.9-21.4)
50 (95% CI)	45.2 (29.5-78.6)	120 (85.5-194)

5.3.1.4 Copper – marine/estuarine

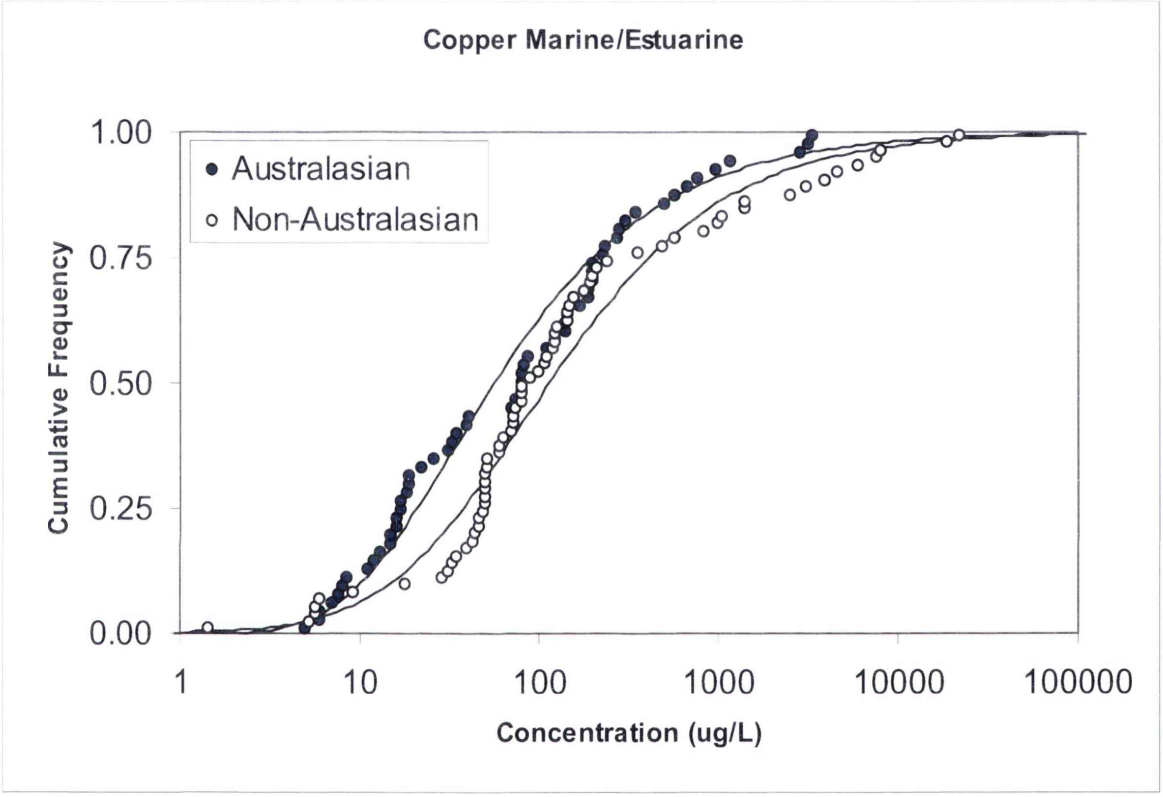

Figure 5.4 Species sensitivity distributions for Australasian and non-Australasian species acutely exposed to Cu in marine/estuarine water.

A total of 59 toxicity data points was used for the Australasian SSD for Cu in marine/estuarine water and 68 were used for the non-Australasian SSD (Figure 5.4). Except for the most extremely sensitive species, the SSD for the Australasian species lies to the left of that for the non-Australasian species indicating that they may be more sensitive. The 95% CIs for the Australasian and non-Australasian PC95 and PC50 values overlap (Table 5.5). The standard error of the difference test (Sprague & Fogels 1977) subsequently indicated that there were no significant differences ($p > 0.05$) between the Australasian and non-Australasian PC95 values and between the PC50

values. Therefore there are no significant differences in the sensitivity of the Australasian and non-Australasian marine/estuarine species to copper.

Table 5.5 Protective Concentrations (PC) for Australasian and non-Australasian species exposed to Cu in marine/estuarine water (µg/L).

PC values	Australasian Acute	Non-Australasian Acute
95 (95% CI)	6.9 (5.2-10.6)	8.4 (4.6-17.5)
50 (95% CI)	56.1 (37.5-87.1)	114 (78.2-176)

5.3.1.5 Zinc – freshwater

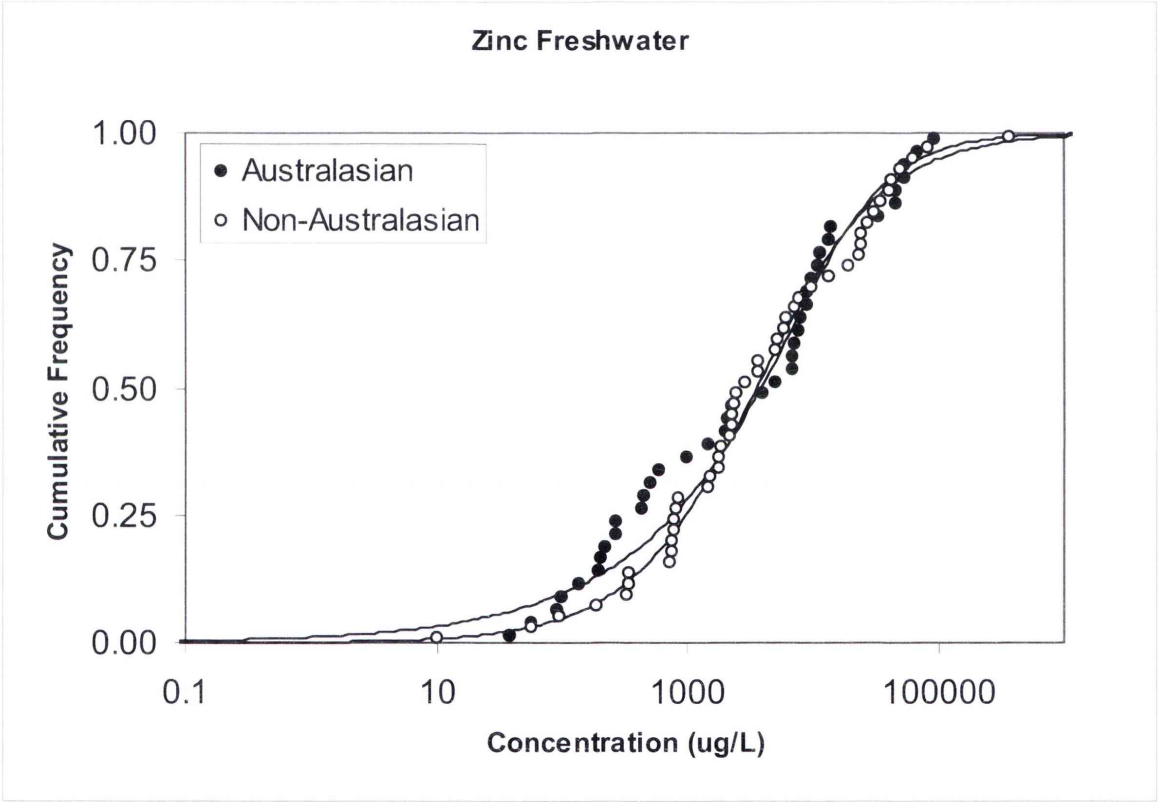

Figure 5.5 Species sensitivity distributions for Australasian and non-Australasian species acutely exposed to Zn in freshwater.

A total of 40 toxicity data points was used for the Australasian SSD for Zn in freshwater and 48 were used for the corresponding non-Australasian SSD (Figure 5.5). Overall there is no real difference in the SSDs of the Australasian and non-Australasian species. The 95% CIs for the Australasian and non-Australasian PC95 and PC50 values overlap (Table 5.6). The standard error of the difference test (Sprague & Fogels 1977) indicated that there were no significant differences ($p > 0.05$) between the Australasian and non-Australasian PC95 values and between the PC50 values. Thus overall there was no

difference in the sensitivity of the Australasian and non-Australasian freshwater species to zinc.

Table 5.6 Protective Concentrations (PC) for Australasian and non-Australasian species exposed to Zn in freshwater (µg/L).

PC values	Australasian Acute	Non-Australasian Acute
95 (95% CI)	25.1 (1.89-132)	106 (19.9-401)
50 (95% CI)	3810 (1020-9960)	3510 (1980-9065)

5.3.1.6 Zinc – marine/estuarine

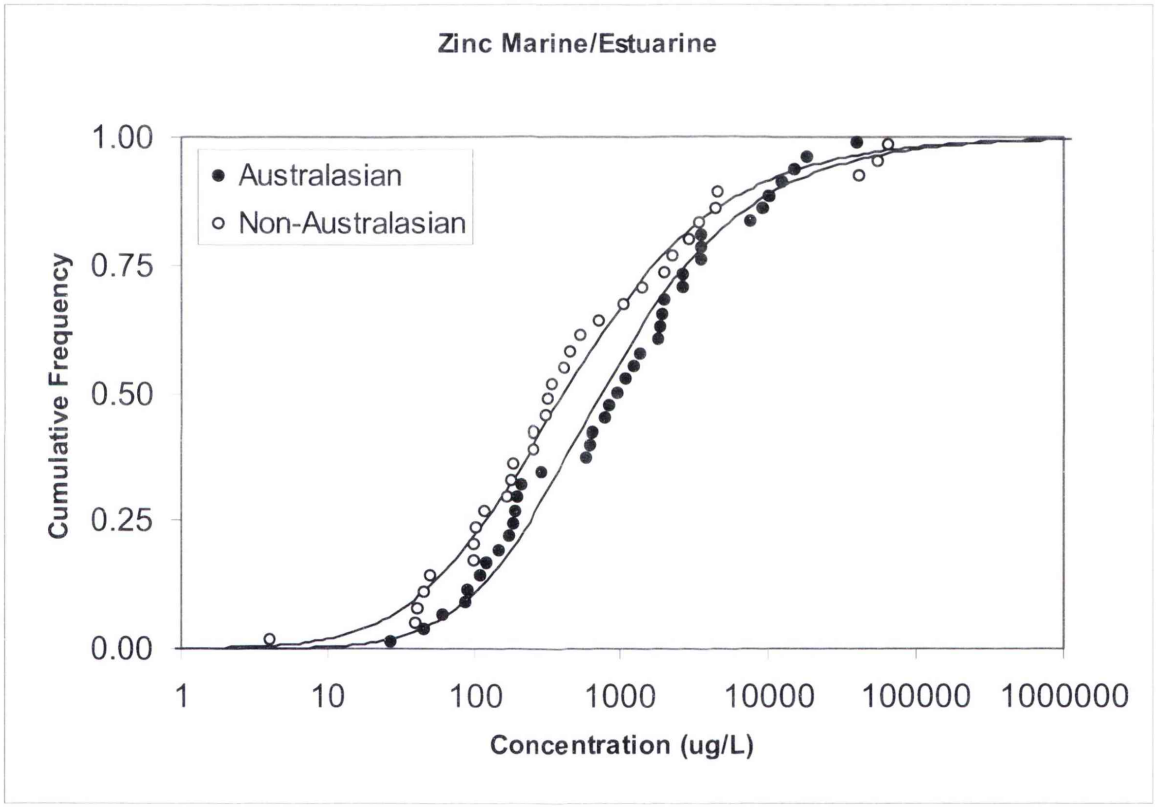

Figure 5.6 Species sensitivity distributions for Australasian and non-Australasian species acutely exposed to Zn in marine/estuarine water.

A total of 39 toxicity data points was used for the Australasian SSD for Zn in marine/estuarine water and 32 were used for the non-Australasian SSD (Figure 5.6). Overall the SSD for the non-Australasian species lies to the left of that for the Australasian species indicating that they may be more sensitive. The 95% CIs for the Australasian and non-Australasian PC95 values and for the PC50 values overlap (Table 5.7). The standard error of the difference test (Sprague & Fogels 1977) indicated that there were no significant differences ($p > 0.05$) between the Australasian and non-

Australasian PC95 values and between the PC50 values. Therefore, overall, there is no difference in the sensitivity of Australasian and non-Australasian marine/estuarine species to zinc.

Table 5.7 Protective Concentrations (PC) for Australasian and non-Australasian species exposed to Zn in marine/estuarine water (µg/L).

PC values	Australasian Acute	Non-Australasian Acute
95 (95% CI)	53 (18-104)	21.4 (7.5-68.5)
50 (95% CI)	737 (386-2100)	407 (216-926)

5.3.2 Comparisons of Australasian and non-Australasian PC values

5.3.2.1 Freshwater

A direct comparison of the estimated chronic trigger values (TVs) for Australasian and non-Australasian species generated from the SSDs for cadmium, copper and zinc in freshwater are presented in Table 5.8. The Australasian species were found to be approximately 2.7 to 4.2 times more sensitive to copper and zinc respectively than the non-Australasian species while the non-Australasian species were found to be approximately 5.5 times more sensitive to cadmium than the Australasian species. Using non-overlapping 95% confidence intervals as a measure of differences being significant, the sensitivity of Australasian species to copper is significantly greater than that for the non-Australasian species, while cadmium and zinc have overlapping 95% CIs. When the standard error of the difference test (Sprague & Fogels 1977) was applied the sensitivity of the non-Australasian species to cadmium was significantly greater than the Australasian species. However, there was no significant difference in the sensitivity of the Australasian and non-Australasian freshwater species to zinc.

Table 5.8 Comparison of estimated chronic trigger values (µg/L) from Australasian and non-Australasian aquatic toxicity data for Cd, Cu and Zn in freshwater.

Metal	Australasian Estimated Chronic TV	Non-Australasian Estimated Chronic TV	Standard Error of the Difference Test Result
Cadmium	0.44 (0.17 – 1.2)	0.08 (0.02 – 0.28)	Non-A[1]
Copper	0.85 (0.53 – 1.4)	2.3 (1.6 – 3.5)	Aust[2]
Zinc	5.7 (0.43 – 30)	24.2 (4.5 – 91.2)	NSD[3]

[1]Non-A = non-Australasian taxa more sensitive; [2] Aust = Australasian taxa more sensitive; [3] NSD = No Significant Difference ($p > 0.05$) detected.

5.3.2.2 Marine/estuarine

A direct comparison of the estimated chronic TVs for Australasian and non-Australasian species generated from the SSDs for cadmium, copper and zinc in marine/estuarine water are presented in Table 5.9. The Australasian species were found to be approximately 2 and 2.5 more sensitive to cadmium and zinc respectively than the non-Australasian species while the non-Australasian species were slightly more sensitive to copper. The standard error of the difference test indicated that the estimated chronic TVs based on Australasian and non-Australasian species were not significantly different for any of the three metals.

Table 5.9 Comparison of estimated chronic trigger values (µg/L) from Australasian and non-Australasian aquatic toxicity data for Cd, Cu and Zn in marine/estuarine water.

Metal	Australasian Estimated Chronic TV	Non-Australasian Estimated Chronic TV	Standard Error of the Difference Test Result
Cadmium	1.5 (0.5 – 5.1)	0.76 (0.32 – 1.6)	NSD[1]
Copper	1.1 (0.85 – 1.7)	1.4 (0.7 – 2.9)	NSD
Zinc	12.1 (4.1 – 23.6)	4.9 (1.7 – 15.6)	NSD

[1] NSD = No Significant Difference ($p > 0.05$) detected.

5.4 DISCUSSION

Species sensitivity distributions (SSDs) have been used by a number of national authorities to derive their WQG TVs (USEPA 1986, Van de Plassche *et al.* 1993, Petersen & Pedersen 1995, Roux *et al.* 1996, ANZECC & ARMCANZ 2000). In this chapter, the BurrliOZ SSD method was used to derive TVs using the data from the Australasian Ecotoxicity Database (Warne *et al.* 1998, Warne & Westbury 1999, Markich *et al.* 2002) and the non-Australasian Ecotoxicity database generated as part of this study. The differences seen between SSDs generated from the two sets of data may indicate greater sensitivity of one group, but there are many factors that may have a bearing on the generated TVs. Species selection, differences in testing methodology, data availability and data quality and choice of SSD approach have all been suggested and investigated as possible sources of influence on any observed differences detected between the two regions (Maltby *et al.* 2005, Kwok *et al.* 2007). The freshwater and marine/estuarine SSDs derived in this study used data points from at least 31 species from 4 taxa with up to 9 taxa and 69 species being used. The data used for the

derivation of these SSDs was subjected to the quality assessment process described in chapter 2 and tested in chapter 3, where the quality, including the methodology used to generate the data, are examined and only included if of acceptable standard. The SSD approach in this chapter was that used to derive the ANZECC/ARMCANZ WQG (2000) TVs with the suitability of the data assessed to ensure the application of this method was appropriate. But, despite its widespread use, the use and merits of the SSD approach has been discussed and debated (e.g. Forbes & Forbes 1993, Smith & Cairns 1993 Schudoma 1994, Maltby *et al.* 2005).

5.4.1 Comparisons with other studies

When the PC95 and PC50 values based on acute toxicity data to Australasian and non-Australasian species for both freshwater and marine/estuarine ecosystems were compared only one significantly different result was detected (i.e. copper in freshwater) . In comparison when the same data were used to estimate chronic trigger values, significant differences were found between the Australasian and non-Australasian species for both copper and cadmium in freshwater. Significant differences were not found between the remaining nine comparisons. If non-Australasian copper data were used to generate trigger values for copper for Australasian freshwater ecosystems, they would not be adequately protected by a factor of 2.7 and if cadmium data were used they would be overprotected by a factor of 5.5.

The minimum number of species that were used for either the Australasian or non-Australasian SSDs was 32, with the largest number being 77. Given these numbers are all greater than those indicated by researchers of being necessary to obtain reliable results from SSDs (i.e. 15 – 30) (Newman *et al.* 2000; Wheeler *et al.* 2002; Forbes and Calow 2002) we can be confident of the PC95 and PC50 values and of the resulting conclusions regarding the relative sensitivity of the species.

The extrapolation factor determined by Kwok *et al.* (2007) for the adequate protection of tropical species from compounds using temperate species data was 10. Therefore WQGs derived using temperate toxicity data were to be divided by 10 in order to protect tropical aquatic ecosystems. The data generated in this study was not really

suitable for a similar analysis as the vast majority of comparisons showed no significant differences. If such an analysis was done, the result would likely show that an assessment factor is very close to one as the two values that differed from one were similar (i.e. 2.7 and 4.2) but were opposite (i.e. one indicated the Australasian species were more sensitive while the other indicated that the non-Australasian species were more sensitive).

The fact that we have comparisons indicating that the Australasian species have the same, lower and greater sensitivity than the non-Australasian species, agrees with those undertaken by other Australian researchers that were discussed in Section 1.3. The results from these studies indicate that there is no overall pattern to the relative sensitivity of the different sets of species which was echoed in the findings of the current investigation.

5.4.2 Comparison of PC values with the Australian and New Zealand WQG TVs

The ANZECC WQG trigger values (TVs) for cadmium, copper and zinc are presented in Table 5.10 (freshwater) and Table 5.11 (marine/estuarine) where they are compared to estimated chronic TVs derived using acute toxicity data for taxa from Australasia, non-Australasia and a combination of the two. These results indicate that using acute to chronic ratios (ACRs) does not always result in similar trigger values. In other words the ACRs are not good at converting acute toxicity data to chronic data. This most likely reflects that each species has its own unique ACR, but in converting acute toxicity data to chronic values usually a single ACR is used for all species. This single value is either: a value for a single species; or a mean (arithmetic or geometric) of ACRs. This finding supports the position adopted in the Australian and New Zealand WQGs (ANZECC & ARMCANZ 2000) and others (e.g. Van de Plasche *et al.* 1993; OECD 1995; Petersen and Pedersen 1995) for where chronic toxicity data are used to derive WQGs in preference to acute toxicity data.

These tables (Table 5.10 and Table 5.11) also present the estimated chronic trigger values based on the Australasian acute toxicity data, non-Australasian acute toxicity data and the Australasian and non-Australasian acute toxicity data combined. There are

a number of cases where the estimated chronic trigger values are up to six times larger or smaller than the current Australian and New Zealand trigger values. This could reflect the smaller datasets used to derive the Australasian and the non-Australasian estimated trigger values compared to the Australian and New Zealand trigger values. However, the estimated trigger values based on both the Australasian and non-Australasian datasets, which have data for many species, still vary markedly around the Australian and New Zealand trigger values.

Table 5.10 Comparison of estimated chronic trigger values derived using Australasian data with the Australian and New Zealand Water Quality Guidelines trigger values for Cd, Cu and Zn in freshwater (µg/L).

Metal	Australasian Estimated Chronic TV	Non-Australasian Estimated Chronic TV	Combined Estimated Chronic TV	WQG TV
Cadmium	0.44 (0.17 – 1.2)	0.08 (0.02 – 0.28)	0.15 (0.063 – 0.38)	0.2
Copper	0.85 (0.53 – 1.4)	2.3 (1.6 – 3.5)	1.29 (0.98 – 1.85)	1.4
Zinc	5.7 (0.43 – 30)	24.2 (4.5 – 91.2)	14.7 (4.11 – 38.33)	8

Table 5.11 Comparison of estimated chronic trigger values derived using Australasian data with the Australian and New Zealand Water Quality Guidelines trigger values for Cd, Cu and Zn in marine/estuarine water (µg/L).

Metal	Australasian Estimated Chronic TV	Non-Australasian Estimated Chronic TV	Combined Estimated Chronic TV	WQG TV
Cadmium	1.5 (0.5 – 5.1)	0.76 (0.32 – 1.6)	0.87 (0.47 – 1.82)	5.5
Copper	1.1 (0.85 – 1.7)	1.4 (0.7 – 2.9)	1.16 (0.83 – 1.79)	1.3
Zinc	12.1 (4.1 – 23.6)	4.9 (1.7 – 15.6)	7.5 (3.72 – 15.25)	15

5.5 CONCLUSIONS

- Species sensitivity distributions could be derived for cadmium, copper and zinc in both fresh and marine/estuarine waters.
- The number of phylum and species used for each SSD was such that the confidence in the derived TV's was high.
- The PC95 and PC50 values based on acute toxicity data for Australasian freshwater species were significantly more sensitive to copper than their non-Australasian counterparts. The remaining five comparisons found no significant differences.
- The estimated chronic trigger values (i.e. PC95 values) for Australasian freshwater species to copper and cadmium were significantly more (i.e. 2.7 times) and less (i.e. 5.5 times) sensitive, respectively, than their non-Australasian counterparts. The remaining four comparisons found no significant differences.
- Acute to chronic ratios are not accurate and reliable means of converting acute data to chronic trigger values.
- Given that there are significant differences in the sensitivity of Australasian and non-Australasian species to copper and cadmium for acute toxicity data it is highly likely that there are differences also between chronic toxicity data.

6 GENERAL DISCUSSION

Ecological hazard and risk assessments and the generation of environmental quality guidelines all rely on ecotoxicity data. The outcomes of the above processes cannot be better than the quality of the ecotoxicity data that they use, because essentially these processes are manipulations of data. Most of these methods manipulate the data so that a single estimate of the sensitivity of each species is used. The use of poor quality data may lead to marked changes in the estimate of a species' sensitivity, particularly when there is limited data available for a species, or the poor data is markedly different to the other values. This could occur even though the geometric mean is used to derive the value for each species. Use of the geometric mean decreases the effect of very large or very small values on the mean. It is therefore imperative that high quality data should be used. This is acknowledged by many regulatory authorities and risk assessors (e.g. ANZECC and ARMCANZ 2000, CCME 1999). For example, the Dutch Government states that they assessed the quality of the available ecotoxicity data and only used what they considered to be of acceptable quality (e.g. Van de Plassche *et al.* 1993). The USEPA in their ECOTOX database (USEPA 2004) (previously called AQUIRE) have a data quality assessment scheme and each datum has been awarded a quality ranking of either complete, moderate or unreliable based on the scores awarded for a series of questions. Environment Canada is also in the process of developing an ecotoxicity data quality assessment scheme (Elpi Karalis, Environment Canada, *pers. comm.*). Before commencing the Australasian Ecotoxicity Database (AED) its authors (Warne *et al.* 1998, Warne and Westbury 1999, Markich *et al.* 2002) examined the literature and found that the only published ecotoxicity data assessment scheme was that in the USEPA ECOTOX database. However, this scheme was not considered to fully assess all the key characteristics of the quality of ecotoxicity data and was extensively modified (Warne *et al.* 1998, Warne and Westbury 1999, Markich *et al.* 2002) as illustrated by Tables 6.1 and 3.3. All the data entered into the AED were assessed using the modified and improved USEPA data quality assessment method. This same method was also used to assess the quality of any ecotoxicity data that had not previously been assessed by a regulatory authority as acceptable, in deriving the Australian and New Zealand Water Quality Guidelines (ANZECC & ARMCANZ 2000).

Table 6.1. The questions used and the marks awarded to determine the quality score and quality class of the toxicity data. Modified from the USEPA (1994).

Question	Possible Marks[1]
Was the duration of the exposure stated?	20 or 0
Were there appropriate controls (e.g. a solvent control if solvents are used)?	5 or 0
Were the characteristics of the test organism stated?	5 or 0
Were the chemical concentrations measured?	5 or 0
Was the type of exposure (e.g. static, flow through) stated?	5 or 0
Was the test location stated?	4 or 0
Was the grade or purity of the test chemical stated?	4 or 0
Was the type of test media used stated?	4 or 0
Was the hardness (for freshwater) or the salinity (for saltwater) measured and stated?	2 or 0
Was the alkalinity (for freshwater) or salinity (for saltwater) measured and stated?	2 or 0
Was the dissolved oxygen content of the test water measured at some stage during or after the test?	2 or 0
Was the temperature measured during the test?	2 or 0
Was the pH of the test water measured at some time during the test?	2 or 0
Was the biological endpoint clearly defined?	20 or 0
Was there a concentration-response relationship either observable or stated?	5 or 0
Was the biological effect quantified i.e. 50% effect, 25% effect?	5 or 0
Was the statistical level of significance for any statistical tests stated (for NOEC/LOEC data)? Was a valid model used to derive the LC50/EC50 values (for LC/EC data)?	4 or 0
Was the stated significance level 0.05 or less (for NOEC/LOEC data)? Was there an estimate of the variability of the LC50 or EC50 (for LC/EC data)?	4 or 0
Total Score	
Class (C, M, I)	

[1] There are only two marks that can be awarded in answering a question – the full mark or zero.

In undertaking this study, metal ecotoxicity data for Australasian and non-Australasian species were collated. The majority of the Australasian ecotoxicity data were obtained from the AED (Markich *et al.* 2002), however, some additional data were added. Since the author of this study was different to the assessors of the metal ecotoxicity data in the AED, it was decided to investigate whether different assessors would give the same assessment of the quality of ecotoxicity data. A preliminary study indicated that differences could occur. It was therefore decided that a larger study involving numerous assessors with different levels of ecotoxicological experience should be conducted. The results of this study were presented in Chapter 3 of this thesis.

This study indicated that there was considerable variation in an assessor's estimate of the quality of the same data. Subsequent analysis indicated that most of the variation in the assessments arose from a number of questions. It was decided to rephrase these

questions and run the assessment again. Revisions of the data quality assessment scheme led to a significant decrease in the variability of the assessors' estimates of the data quality. Despite these improvements there were still differences in the assessed quality of the data. However, the number of instances where assessors gave such widely differing assessments of quality that it changed the classification, and therefore whether the data could be used or not, were very limited. This finding illustrates the relative robustness of the data assessment scheme and that most scientists with ecotoxicology knowledge can assess data quality.

Care needs to be exercised when assessing the quality of ecotoxicity data. One of the key criticisms of species sensitivity distribution methods (SSDs) such as the BurrliOZ method (Campbell *et al.* 2000) used in this study, is that they are often used for chemicals with toxicity data for as little as five species that belong to four taxonomic groups (Van de Plassche *et al.* 1993, OECD 1995, Petersen and Pedersen 1995, ANZECC and ARMCANZ 2000). It has been argued that such small datasets cannot be representative of all organisms in the ecosystems being protected and that the use of such small datasets leads to the generation of highly variable trigger values (e.g. Forbes and Calow 2002). For example, Newman *et al.* (2000) showed that the variability of trigger values is minimal when there are toxicity data for between 15 and 55 species, with a mean of 30 species. Other authors including Wheeler *et al.* (2002) suggested that 15 - 25 species were needed, while Forbes and Calow (2002) used empirical techniques to estimate that at least 20 species are needed to estimate a PC95. Kefford *et al.* (2005) argued that the majority of species for which there are toxicity data are common species and that there are very little toxicity data for rarer species. They also argued and cited published examples, that there are, at least in some instances, significant differences in the relative sensitivity of rare and common species. They therefore argued that it is better to have data for more species, perhaps of lower precision, used in a SSD method than it is to have more precise data for fewer species. They advocated that less precise methods such as the 'up and down' method (e.g. Sunderam *et al.* 2004) which also use far fewer test organisms than traditional toxicity test methods, should be used to provide toxicity data for more species. Further support for this argument comes from the study by Sunderam *et al.* (2004) which showed that in the vast majority of the comparisons they made, there were no significant differences between the EC50 values derived using traditional methods and the 'up and down' method. This suggests that such methods

may not be any less precise than data derived from traditional methods. Even if the 'up and down' method is used to generate toxicity data, the resulting toxicity data will need to have their quality assessed.

As mentioned earlier, for the majority of chemicals there is ecotoxicity data for a limited range of species. The number of species for which there are data can be further reduced due to data quality procedures (e.g. Wheeler *et al.* 2002). Kefford *et al.* (2004) and Wheeler *et al.* (2002) argue that data quality assessment procedures will not randomly remove species, rather they will predominantly remove data from non-standard organisms and could therefore be introducing a bias in the data used in SSDs.

Ecotoxicity data assessment methods should be designed in such a manner as to not unnecessarily favour traditional methods if equally rigorous alternative methods are used. It would also appear sensible, given that many nations use data for species from other countries/regions, to develop an internationally acceptable method for assessing toxicity data. The most logical venues for this are the OECD, the United Nations, one of the internationally recognised regulatory authorities or a scientific organisation such as the Society of Environmental Toxicology and Chemistry (SETAC).

Kwok *et al.* (2007) calculated the ratio of the sensitivity of tropical and temperate aquatic species and then plotted the cumulative frequency of these against the ratio. From this, they determined if it was desired to protect 95% of species, then an assessment factor of 10 was required for chemicals that had never been assessed using tropical species. Using the same process the ratios of the sensitivity of non-Australasian species to the Australasian species from the student t-test chapter (Chapter 4) were placed in ascending order and then ranked. The cumulative frequency for each ratio was determined using the formula

$$\text{Cumulative Percent} = [\text{rank} / (n+1)] \times 100$$

where n is the number of data points in each set of data being compared. The ratios of toxicity (y axis) and their corresponding cumulative frequencies (x axis) were then plotted separately for the freshwater species and for the marine/estuarine species (see Figure 6.1 and Figure 6.2 respectively). A similar analysis using ratios of the sensitivity

of Australasian and non-Australasian species from the SSD chapter (Chapter 5) was not conducted as it would have little meaning as there were only three data points for each comparison.

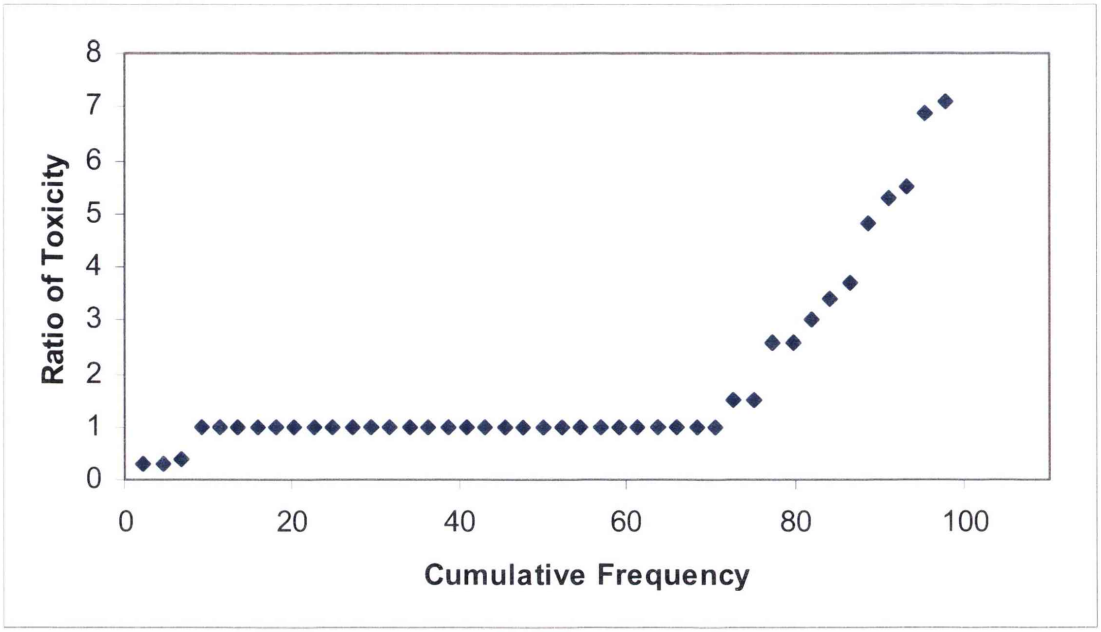

Figure 6.1 Cumulative frequencies of the ratio of toxicity of non-Australasian to Australasian species exposed to metals in freshwater.

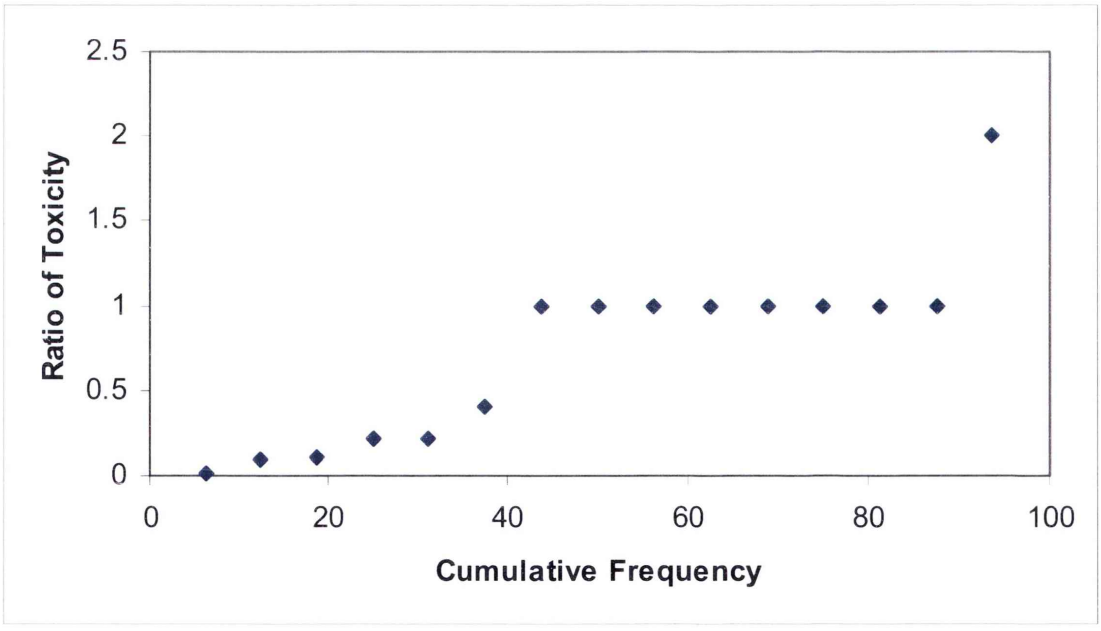

Figure 6.2 Cumulative frequencies of the ratio of toxicity of non-Australasian to Australasian species exposed to metals in marine/estuarine water.

The majority of the ratios in Figure 6.1 and Figure 6.2 had values of one, therefore they were replotted using only a single ratio equal to one and all ratios greater than one (see Figure 6.3 and Figure 6.4). These plots were then subjected to simple linear regression

and the resulting equations used to determine the ratio of toxicity at various degrees of protection (Table 6.2). The values presented in the second and fourth column of Table 6.2 are the safety factors that would need to divide the non-Australasian data by in order to protect 95% of Australasian species in fresh and marine/estuarine water respectively, from various percentages of chemicals.

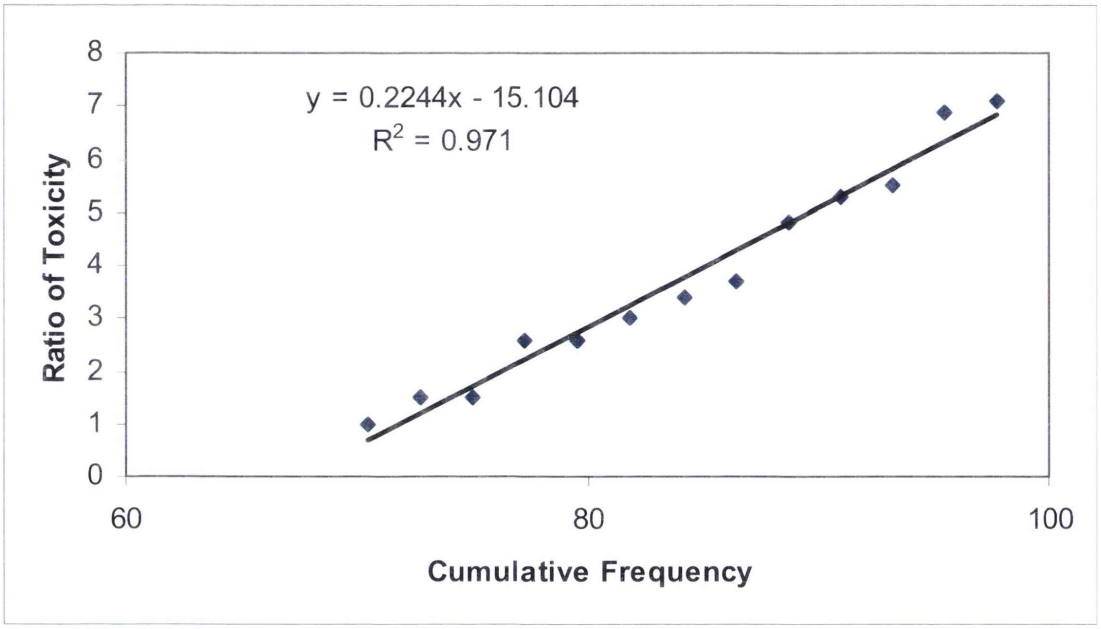

Figure 6.3 Cumulative frequencies of the ratio of toxicity of non-Australasian to Australasian species exposed to metals in freshwater for chemicals with ratios greater than one.

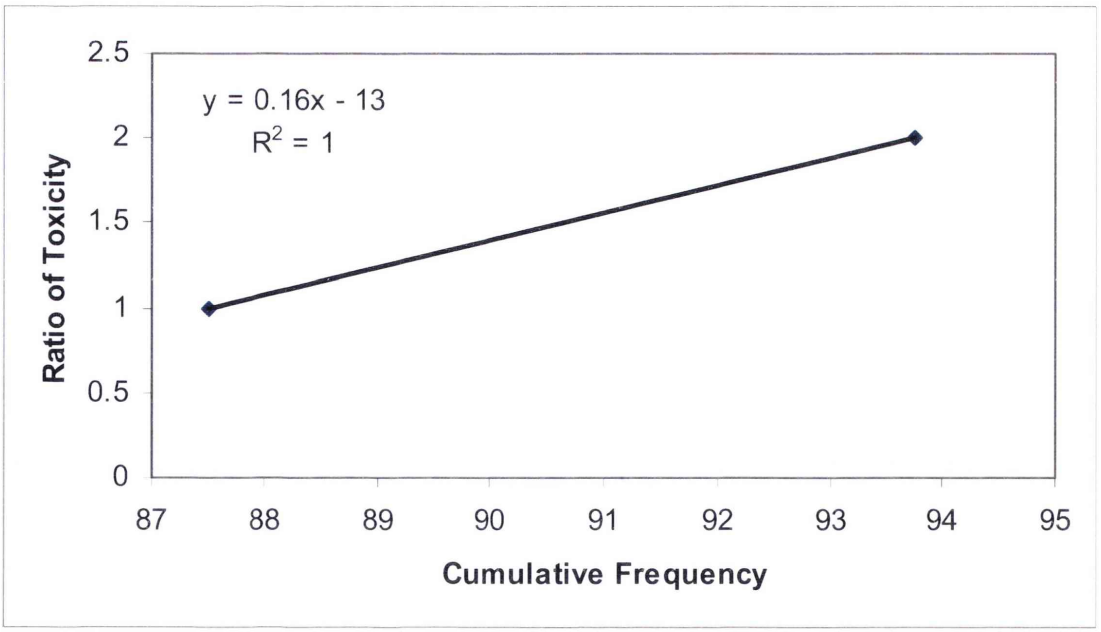

Figure 6.4 Cumulative frequencies of the ratio of toxicity of non-Australasian to Australasian species exposed to metals in marine/estuarine water for chemicals with ratios greater than one.

Table 6.2. Safety factors that would be needed to protect 95% of Australasian species from varying percentages of chemicals, using non-Australasian toxicity data.

Freshwater		Marine/estuarine water	
Degree of Protection (% of chemicals)	Safety Factor	Degree of Protection (% of chemicals)	Safety Factor
80	2.85	80	-0.20
85	3.97	85	0.60
90	5.09	90	1.40
95	6.21	95	2.20
99	7.11	99	2.84

It would be consistent with the level of species protected, if the Australasian species were protected from 95% of the chemicals. Thus the safety factors that non-Australasian toxicity data would need to be divided by in order to protect Australasian freshwater and Australasian marine/estuarine species from 95% of chemicals are 6.2 and 2.2 respectively. The values obtained in this study for both freshwater and marine/estuarine species fall within the range of values obtained by studies (2 to 14) that compared the sensitivity of organisms from different geographical or ecological regions (e.g. Maltby *et al.* 2005, Kwok *et al.* 2007).

While differences between safety factors for freshwater and marine/estuarine species obtained by this study are not large, they are still of sufficient size to warrant explanation. It is possible that many of the marine/estuarine organisms in Australasia and non-Australasia have not been isolated from each other for long periods of time – as there are no real barriers impeding movement and the exchange of genetic material. In contrast, the Australasian freshwater species and those of non-Australasia (predominantly North America and Europe) are isolated currently from each other by vast oceans and in addition, they have been isolated for long geological periods. It is therefore highly likely that the freshwater species in the two regions are more biogeographically different than the marine species and this is reflected in the values of the safety factors for the freshwater and marine/estuarine species.

Different statistical techniques were used to make comparisons of the sensitivity of Australasian and non-Australasian species to metals in Chapters 4 and 5. Chapter 4 used the 95% confidence intervals and Student's t-test, while Chapter 5 used an SSD method. These methods have different minimum data requirements. The Student's t-tests were

only used when there was a minimum of three data points for the Australasian and for the non-Australasian groups of organisms being compared (e.g. organisms belonging to the same taxonomic group, or same species or equivalent species). In comparison, the SSD method requires data from at least five species that belong to at least four taxonomic groups. The Student's t-test compares the means of two groups to see if they are different, while the SSD method compares the distribution of species sensitivity by comparing the concentrations that should theoretically protect selected percentages of species. The closest possible comparison that could be made using the Student's 't' method to those made by the SSD method would be a comparison of the means of all the Australasian data and non-Australasian data for each metal. However, this particular comparison was not made as it was considered to be a meaningless comparison and highly unlikely to result in a meaningful answer, since the variance around the means for each group would be so large that no significant differences were likely to be found.

Due to the necessary data requirements for constructing SSDs, the number of comparisons between Australasian and non-Australasian organisms that could be undertaken using this method was limited, compared to the number of comparisons that were available using the t-test method.

Comparisons of results from Chapter 4 and Chapter 5 cannot be made directly due to SSDs using data for all species to generate the estimated chronic TVs while the t-tests evaluated sensitivity differences using specific data sets for phyla and species comparisons. T-tests incorporating all phyla and using the different subsets of data as used in Chapter 4 (EC/LC50, EC50, LC50) were used to make comparisons between the two statistical methods. The results of these comparisons are displayed in Table 6.3.

The results for copper comparisons in both fresh and marine/estuarine water agree well between the two statistical methods. For the freshwater comparisons, the SSD comparison found that Australasian species were significantly more sensitive, which was also found in all t-test comparisons. The marine/estuarine SSD for copper indicated that there was no significant difference between the Australasian and non-Australasian species which was mirrored in two of the three t-test comparisons with only the LC50 comparison indicating the non-Australasian species being significantly more sensitive.

Table 6.3. Summary of the results of the comparisons of the sensitivity of Australasian and non-Australasian taxa using estimated chronic trigger values and t-test results.

	Freshwater				Marine Estuarine			
	SSD	t-test			SSD	t-test		
Metal		EC/LC50	EC50	LC50		EC/LC50	EC50	LC50
Copper	Aust[3]	Aust	Aust	Aust	NSD	NSD	NSD	Non-A
Cadmium	Non-A[1]	NSD[2]	NSD	NSD	NSD	Non-A	NSD	Non-A
Zinc	NSD	Aust	Non-A	Aust	NSD	Non-A	Non-A	Non-A

[1] Non-A = non-Australasian taxa more sensitive; [2] NSD = No Significant Difference ($p > 0.05$) detected using Students t-test; [3] Aust = Australasian taxa more sensitive.

The cadmium in freshwater, and the zinc in marine/estuarine water comparisons are interesting in that both results for the SSD comparison did not agree with the any of the t-test results. The cadmium in freshwater SSD found that the non-Australasian organisms were significantly more sensitive, but all three t-test comparisons indicated that there were no significant differences between the two sets of organisms. The difference detected between the Australasian and non-Australasian SSD for cadmium was not evident from the 95% confidence intervals, as they overlapped, but when the standard error of the difference test was used, a significant difference was found. This indicates that the difference between the two sets of organisms is only small and was not detected by the t-tests. The SSD comparison for zinc in marine/estuarine water is not significantly different, but all the t-tests indicate that the Non-Australasian species are significantly more sensitive. The non-Australasian PC95 is over two times lower than the Australasian PC95, it is therefore likely that the distributions were very close to being significantly different at the 0.05 level.

The t-test comparisons for cadmium in marine/estuarine water yielded variable results with the EC/LC50 data and LC50 data finding the non-Australasian organisms significantly more sensitive, but the EC50 data found no significant difference, which agreed with the finding of the SSD comparison. The SSD comparison for zinc in freshwater found no significant difference between the two groups of organisms, but EC/LC50 and LC50 t-test comparisons indicated that the Australasian organisms were significantly more sensitive while the EC50 data indicated that the non-Australasian organisms were more sensitive.

In approximately 33% of the comparisons of the SSD and t-test results there was agreement between the results. However, due to the methods testing different hypotheses and having different data requirements the results of Chapters 4 and 5 are not directly comparable but rather they are complimentary and reveal different things about the relative sensitivity of Australasian and non-Australasian species to metals.

In Chapter 4 it was found that when using the most reliable data set available for each combination of the 37 freshwater comparisons, 40% of the comparisons were significantly different. Of those that were found to be significantly different, the Australasian taxa were more sensitive in 80% of the cases. The ratios of the sensitivity of all non-Australasian and Australasian taxa indicated that the Australasian species were significantly more sensitive than the non-Australasian species.

When using the most reliable data set available for each combination of the 15 marine/estuarine comparisons, 47% of the comparisons were significantly different. Of those that were found to be significantly different, the non-Australasian taxa were more sensitive in 86% of the cases. When using ratios of the sensitivity of all non-Australasian and Australasian taxa it was found the non-Australasian marine/estuarine species were not significantly more sensitive than the corresponding Australasian species. If non-Australasian species toxicity data were used to protect Australasian ecosystems, freshwater ecosystems would not be adequately protected, but marine/estuarine ecosystems would be adequately protected.

The results of Chapter 4 tell us whether there are differences in the sensitivity of comparable groups of Australasian and non-Australasian aquatic organisms. Such information could be of immense value when trying to estimate the effect of a metal on a group or species of the Australasian biota and there is a lack of available data, but there is data for northern hemisphere groups or species. It is clear from this chapter that some species and some groups of organisms in Australasia and non-Australasia have different sensitivities to metals. However, in environmental protection we are almost invariably concerned with protecting assemblages of species and ecosystems rather than individual species or individual types of organisms. This is reflected in the WQG that many countries have adopted. In Australia and New Zealand for example, the aim is to protect 95% of species in slightly to moderately modified aquatic ecosystems.

Therefore, the question from an environmental protection point of view is not so much 'are there differences in the sensitivity of individual species or groups of organisms' but rather 'do these change the WQG'. This distinct difference between a statistical difference and a biologically meaningful difference in sensitivity was realised by Phyu *et al.* (*in press*). They found significant ($p < 0.05$) differences in the toxicity of atrazine and molinate to a suite of six aquatic species when tested in water only and water plus sediment test conditions. Yet when they used this data in SSDs they found there were no significant ($p > 0.05$) differences in the resulting PC95 values. It is therefore, quite possible for there to be significant differences in the sensitivity of Australasian species or types of organisms and yet be no change in the resulting WQG. This could occur because the differences between species or types of organisms may cancel each other out (i.e. in some cases the Australasian are the more sensitive and in other cases the non-Australasian are the more sensitive). The results of the SSD chapter (Chapter 5) provide us with the answer to this question.

It is a great shame that the lack of available toxicity data results in so few chemicals (i.e. Cu, Cd and Zinc in freshwater and marine/estuarine water) meeting the minimum data requirements of the SSD method. Depending on the type of data being compared one or two out of six comparisons exhibited significantly different sensitivities. These translate to 16.66% and 33.33% of the comparisons exhibiting significant differences in sensitivity. Chapter 4, which compared the sensitivity using the student t-test method, found 35% and 47% of the comparisons had significantly different sensitivities. That the comparisons made in Chapter 4 yielded a higher percentage of significant differences than the SSD method is not surprising for the reasons explained above. Given this, the percentages of significant differences obtained in these two chapters are surprisingly consistent.

Given that this study has found not only significant differences in the sensitivity of individual species, but types of organisms and the overall species sensitivity it can be concluded that hazard and risk assessments and licensing agreements conducted in Australia for metals may well have, in some cases, been inaccurate. These inaccuracies may have in some instances lead to inadequate species protection being provided and to higher concentrations of metals being permitted. Conversely, in some cases too high a level of protection may have been provided and the permitted concentrations of metals

may have been unnecessarily low. It would appear pertinent to examine some hazard and risk assessments that have already been conducted within Australia to determine if the observed insensitivity, affect the outcomes.

Furthermore, given that there are significant differences in the sensitivity of some Australasian and non-Australasian species to metals it is highly likely that differences in sensitivity would also occur for other groups of chemicals (e.g. organic chemicals, inorganic chemicals). It would therefore appear pertinent that similar analyses to those conducted in this project, be conducted for other groups of chemicals.

6.1 FURTHER RESEARCH

- The generation of high quality ecotoxicity data is paramount for the derivation and refinement of robust trigger values.
- Investigate the influence of factors such as tropical versus temperate species and the effects of isolation by sea on the difference in sensitivities between the two regions.
- Undertake a similar study to determine the differences in sensitivities of Australasian and non-Australasian species to organic compounds.

7 GENERAL CONCLUSIONS

- The AED toxicity data quality assessment scheme is an effective tool for assessors to confidently, no matter what their degree of ecotoxicological experience, assess and classify the quality of aquatic toxicity data reported in a published or unpublished research paper.
- For t-tests analyses, when using the most reliable dataset available for each combination of 37 freshwater comparisons, 40% were significantly different. Of those that were found to be significantly different, the Australasian taxa were more sensitive in 80% of the cases.
- Ratios of the sensitivity of all non-Australasian and Australasian freshwater taxa indicated that the Australasian species were significantly more sensitive than the non-Australasian species.
- When using the most reliable dataset available for each combination of the 15 marine/estuarine comparisons, 47% were significantly different. Of those that were found to be significantly different, the non-Australasian taxa were more sensitive in 86% of the cases.
- When using ratios of the sensitivity of all non-Australasian and Australasian taxa it was found the non-Australasian marine/estuarine species were not significantly more sensitive than the corresponding Australasian species.
- A species sensitivity distribution method could be used to compare the sensitivity of Australasian and non-Australasian taxa for freshwater and marine/estuarine ecosystems for copper, cadmium and zinc. The PC95 and PC50 values based on acute toxicity data for Australasian freshwater species were significantly more sensitive to copper than their non-Australasian counterparts. The remaining five comparisons found no significant differences.
- The estimated chronic trigger values (i.e. PC95 values) for non-Australasian freshwater species to copper and cadmium were significantly (i.e. 5.5 times) more (i.e. 2.7 times) and less (i.e. 4.2 times) sensitive, respectively, than their Australasian counterparts. The remaining four comparisons found no significant differences.
- Acute to chronic ratios are not an accurate or reliable means of converting acute data to chronic trigger values.

- Given that there are significant differences in the sensitivity of Australasian and non-Australasian species to copper and cadmium for acute toxicity data it is highly likely that there are differences also between chronic toxicity data.
- Results from both the Student t-test and SSD comparisons found very similar percentages of the comparisons were significantly different.
- Given that this study has found not only significant differences in the sensitivity of individual species, but types of organisms and the overall species sensitivity, there is reason to believe that over and under protection of our local ecosystems may be occurring using the current WQG's and hazard and risk assessments that have been conducted in Australia which are based predominantly on non-Australasian toxicity data.
- As significant differences were detected in this study it is highly likely that other chemical groups may exhibit significant differences in sensitivity between organisms from the two regions organisms.
- Therefore it would be pertinent that similar analyses be conducted for other groups of chemicals.

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