The University of Sydney

School of Geosciences

QUANTIFYING ENVIRONMENTAL RISK OF GROUNDWATER CONTAMINATED WITH VOLATILE CHLORINATED HYDROCARBONS

by

JAMES HUNT

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

June 2009

ABSTRACT

Water quality guidelines (WQGs) present concentrations of contaminants that are designed to be protective of aquatic ecosystems. In Australia, guidance for assessment of water quality is provided by the ANZECC and ARMCANZ (2000) Guidelines for Fresh and Marine Water Quality. WQGs are generally provided for individual contaminants, not complex mixtures of chemicals, where interaction between contaminants may occur. Complex mixtures of contaminants are however, more commonly found in the environment than singular chemicals. The likelihood and consequences of adverse effects occurring in aquatic ecosystems resulting from contaminants are generally assessed using an ecological risk assessment (ERA) framework. Ecological risk assessment is often a tiered approach, whereby risks identified in early stages, using conservative assumptions, prompt further detailed and more realistic assessment in higher tiers. The objectives of this study were: to assess and investigate the toxicity of the mixture of volatile chlorinated hydrocarbons (VCHs) in groundwater to indigenous marine organisms; to present a 'best practice' ecological risk assessment of the discharge of contaminated groundwater to an estuarine embayment and to develop techniques to quantify the environmental risk; and to evaluate the existing ANZECC and ARMCANZ (2000) WQGs for VCHs and to derive new WQGs, where appropriate.

Previous investigations at a chemical manufacturing facility in Botany, Sydney, identified several plumes of groundwater contamination with VCHs. Contaminated groundwater containing a complex mixture of VCHs was identified as discharging, via a series of stormwater drains, to surface water in nearby Penrhyn Estuary, an adjacent small intertidal embayment on the northern margin of Botany Bay. A screening level ecological hazard assessment was undertaken using the hazard quotient (HQ) approach, whereby contaminant concentrations measured in the environment were screened against published trigger values (TVs) presented in ANZECC and ARMCANZ (2000). Existing TVs were available for 9 of the 14 VCHs present in surface water in the estuary and new TVs were derived for the remaining 5 VCHs. A greater hazard was identified in the estuary at low tide than high tide or when VCH concentrations from both high and low tides were

assessed together. A greater hazard was also identified in the estuary when the toxicity of the mixture was assessed, rather than the toxicity of individual contaminants. The screening level hazard assessment also identified several limitations, including: the low reliability of the TVs for VCHs provided in ANZECC and ARMCANZ (2000); the limited applicability of the TVs to a complex mixture of 14 potentially interacting contaminants; the use of deterministic measures for each of the exposure and toxicity profiles in the HQ method and the associated lack of elements of probability to assess 'risk'. Subsequent studies were undertaken to address these identified shortcomings of the screening level hazard assessment as described in the following chapters.

A toxicity testing methodology was adapted and evaluated for suitability in preventing loss of VCHs from test solutions and also for testing with 6 indigenous marine organisms, including: oyster (*Saccostrea commercialis*) and sea urchin larvae (*Heliocidaris tuberculata*); a benthic alga (*Nitzschia closterium*); an amphipod (*Allorchestes compressa*); a larval fish (*Macquaria novemaculeata*); and a polychaete worm (*Diopatra dentata*). The study evaluated possible VCH loss from 44 mL vials for small organisms (*H.tuberculata*, *S.commercialis* and *N.closterium*) and 1 L jars for larger organisms (*M.novemaculeata*, *A.compressa* and *D.dentata*). Vials were effective in preventing loss of VCHs, however, an average 46% of VCHs were lost from jars, attributable to the headspace provided in the vessels. Test jars were deemed suitable for use with the organisms as test conditions, i.e. dissolved oxygen content and pH, were maintained, however, variability in test organism survival was identified, with some control tests failing to meet all acceptance criteria.

Direct toxicity assessment (DTA) of groundwater contaminated with VCHs was undertaken using 5 indigenous marine organisms and site-specific species sensitivity distributions (SSDs) and TVs were derived for the complex mixture of VCHs for application to surface water in Penrhyn Estuary. Test organisms included *A.compressa*, *H.tuberculata*, *S.commercialis*, *D.dentata* and *N.closterium*. The SSD was derived using NOEC data in accordance with procedures presented in ANZECC and ARMCANZ (2000) for deriving WQGs. The site-specific SSD adopted was a log-normal distribution, using an

acute to chronic ratio (ACR) of 5, with a 95% TV of 838 µg/L total VCHs. A number of additional scenarios were undertaken to evaluate the effect of including different ACRs (i.e. 5 or 10), inclusion of larval development tests as either acute or chronic tests and choice of SSD distribution (i.e. log-normal, Burr Type III and Pareto). TVs for the scenarios modelled varied from 67 µg/L to 954 µg/L total VCHs.

A site-specific, quantitative ERA was undertaken of the surface water contaminated with VCHs in Penrhyn Estuary. The risk assessment included probabilistic elements for toxicity (i.e. the site-specific SSD) and exposure (i.e. a cumulative distribution function of monitoring data for VCHs in surface waters in the estuary). The joint probability curve (JPC) methodology was used to derive quantitative estimates of ecological risk (δ) and the type of exposure in the source areas in surface water drains entering the estuary, i.e. Springvale and Floodvale Drains, Springvale and Floodvale Tributaries and the Inner and Outer Estuary. The risk of possible adverse effects and likely adverse effects were each assessed using SSDs derived from NOEC and EC50 data, respectively. Estimates of risk (δ) of possible adverse effects (i.e. based on NOEC data) varied from a maximum of 85% in the Springvale Drain source area to <1% in the outer estuary and estimates of likely adverse effects (i.e. based on EC50 data) varied from 78% to 0%. The ERA represents a 'best practice' ecological risk assessment of contamination of an estuary using site-specific probabilistic elements for toxicity and exposure assessments.

The VCHs identified in surface water in Penrhyn Estuary are additive in toxicity and act under the narcotic pathway, inhibiting cellular processes through interference with membrane integrity. Lethal toxicity to 50% of organisms (i.e. LC50) is typically reported at the internal lethal concentration (ILC) or critical body residue (CBR) of ~2.5 mmol/kg wet weight or within the range of 1 to 10 mmol/kg wet weight. To evaluate the sensitivity of the test organisms to VCHs and to determine if toxicity in the DTA was due to VCHs, the internal residue for 6 test organisms was calculated for the mixture of VCHs in groundwater and toxicity testing with seawater spiked individually 2 VCHs, chloroform and 1,2-dichloroethane. Calculated residues (at LC50/EC50) were typically between 1 and

10 mmol/kg, with the exception of the algal and sea urchin toxicity tests, which were considerably lower than the expected minimum. Mean internal residues for the groundwater, chloroform and 1,2-dichloroethane were 0.88 mmol/kg, 2.84 mmol/kg and 2.32 mmol/kg, respectively, i.e. close to the predicted value of ~2.5 mmol/kg, indicating that the organisms were suitably sensitive to VCHs. There was no significant difference ($P>0.05$) between the mean residues of each of the three treatments and the study concluded that the additive toxicity of the VCHs in groundwater was sufficient to account for the observed toxicity (i.e. VCHs caused the toxicity in the DTA undertaken).

Evaluation of the existing low reliability ANZECC and ARMCANZ (2000) TVs for chloroform and 1,2-dichloroethane was undertaken to determine if these guidelines were protective of indigenous marine organisms. NOECs, derived from toxicity testing of 1,2-dichloroethane and chloroform with 6 indigenous marine organisms, were screened against the existing low reliability TVs. The TVs for 1,2-dichloroethane and chloroform were protective of 4 of the 6 species tested (*A.compressa*, *D.dentata*, *S.commercialis* and *M.novemaculeata*), however, the TVs were not protective of the alga (*N.closterium*) or the sea urchin larvae (*H.tuberculata*). As the existing TVs were not considered to be adequately protective, SSDs were derived using the NOEC data generated from the testing in accordance with procedures outlined in ANZECC and ARMCANZ (2000). Moderate reliability TVs of 3 µg/L and 165 µg/L were derived for chloroform and 1,2-dichloroethane, respectively, i.e. considerably lower than the existing TVs of 770 µg/L and 1900 µg/L. Differences between the existing and newly derived TVs were considered to result from the sensitive endpoints selected (i.e. growth and larval development rather than survival) and from variability inherent when deriving SSDs using a small number of test species.

Ongoing groundwater monitoring indicated that the plumes of VCHs in groundwater, identified in the 1990s, were continuing to migrate towards Botany Bay. Discharge of these groundwater plumes into Botany Bay would result in significant increases in the concentrations of VCHs in the receiving environment and would likely lead to significant

environmental impacts. In 2006, a groundwater remediation system was commissioned to prevent the discharge of groundwater containing VCHs into Penrhyn Estuary and Botany Bay. The success of the project had only been measured according to chemical and engineering objectives. Assessment of changes in ecological risk is vital to the success of ERA and central to the ERA management framework. Whereas monitoring of chemical concentrations provides qualitative information that risk should decrease, it cannot quantify the reduction in ecological risk. To assess the ecological risk following implementation of the groundwater treatment system, the risk assessment was revised using surface water monitoring data collected during 2007 and 2008. The ERA indicated that, following remediation of the groundwater, ecological risk in Penrhyn Estuary reduced from a maximum of 35% prior to remediation, to a maximum of only 1.3% after remediation. Using the same methodology applied in the initial risk assessment, the success of the groundwater remediation was measured in terms of ecological risk, rather than engineering or chemical measures of success.

Prior to the present investigation, existing techniques for assessing ecological risk of VCH contamination in aquatic ecosystems were inadequate to characterise ecological risk. The current study demonstrated that through monitoring of surface water at the site and DTA using indigenous marine organisms, ecological risk can be assessed using site-specific, quantitative techniques for a complex mixture of VCHs in groundwater. The present investigation also identified that existing ANZECC and ARMCANZ (2000) low reliability TVs were less protective of indigenous test organisms than previously thought and therefore, new TVs were derived in the current work. The present study showed that revision of the risk assessment as conditions change is crucial to the success of the ecological risk management framework.

DECLARATION

I certify that this thesis entitled “Quantifying environmental risk of groundwater contaminated with volatile chlorinated hydrocarbons” submitted for the degree of Doctor of Philosophy has not been submitted for a higher degree at any other university or institution.

I also certify that the thesis has been written by me and is the result of my own research. Any other assistance I have received has been acknowledged in the thesis.

Signature of candidate…… Date…….

ACKNOWLEDGEMENTS

This work was financially supported by Orica Australia Pty Ltd. Orica's generous assistance enabled the project to proceed. Thanks must go, in particular, to James Fairweather who supported this research project.

My sincerest thanks go to my supervisor, Associate Professor Gavin Birch. I would like to thank Gavin for having the courage to take on a 'part-timer' as a research student and allowing me the freedom to develop my research. Gavin's patience and assistance throughout my candidature greatly reduced the agony of undertaking part-time research. I must also thank Gavin for his guidance and development of my writing skills.

I would also like to sincerely thank Dr Michael Warne of CSIRO Land and Water, my associate supervisor, for his invaluable assistance and supervision. The publications contained within this thesis are considerably improved in both clarity and content as a result of Michael's contributions and generosity in tirelessly reviewing numerous versions of manuscripts.

Thanks must also go to Dr Rick Krassoi, Director of Ecotox Services Australasia Pty Ltd. Rick's knowledge, enthusiasm and guidance during the toxicity testing greatly improved the quality and scope of the work undertaken. The research would not have proceeded without Rick's generosity of both time and laboratory support.

I am also grateful for the support provided by Brian Williams and ALS Environmental for the analysis of endless numbers of samples for volatile chlorinated hydrocarbons and for generous technical assistance over the years.

I greatly appreciate the guidance of Dr Stuart Taylor, who taught me two important lessons. The first, that there are no points for stamp collecting and the second, that the journey, sometimes, is more important than the destination.

To Derek Wyman, thanks must go for also easing the pain associated with navigating the University's and the School's administration.

To my parents, Jennifer and Gregson, I am especially grateful for their unwavering support throughout my education and for providing me with the drive and determination to undertake and complete tasks such as this.

Finally, to my lovely wife, without her understanding and support, I could not have devoted so much time and energy to the undertaking of this thesis and for which I will be eternally grateful.

TABLE OF CONTENTS

GLOSSARY

ACR	Acute to Chronic Ratio
AF	Assessment Factor
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
ANZECC	Australia and New Zealand Environment and Conservation Council
ARMCANZ	Agriculture and Resource Management Council of Australia and New Zealand
AR grade	Analytical Reagent Grade
ASW	Artificial Salt Water
AUC	Area Under the Curve
BCF	Bioconcentration Factor
CA	Concentration Addition
CBR	Critical Body Residue
CDF	Cumulative Distribution Function
CI	Confidence Interval
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DTA	Direct Toxicity Assessment
EC*x*	Effect Concentration to *x* percentage of species i.e. EC20 equates to an adverse effect to 20% of test organisms.
ELS	Early Life Stage
ERA	Ecological Risk Assessment
EU	European Union
FSW	Filtered Salt Water
FVD	Floodvale Drain
FVT	Floodvale Tributary
GC/MS	Gas Chromatography-Mass Spectrometry
GTP	Groundwater Treatment Plant
ha	hectare

HC*x*	Hazardous Concentration to x percentage of species. For example, HC5 equates to a Hazardous Concentration to 5% of species. It is the converse of PC95, a concentration that is theoretically protective of 95% of species.
HQ	Hazard Quotient
IE	Inner Estuary
IEC	Internal Effect Concentration
ILC	Internal Lethal Concentration
ISO	International Organization for Standardization
JPC	Joint Probability Curve
K_{ow}	Octanol water partition coefficient
K_{oc}	Organic Carbon partition coefficient
LC*x*	Lethal Concentration to *x* percentage of species i.e. LC50 equates to a lethal response in 50% of test organisms.
LOEC	Lowest Observed Effect Concentration
LOR	Limit of Reporting
NaDS	Sodium dodecyl sulfate
NOEC	No Observed Effect Concentration
NSW	New South Wales
OE	Outer Estuary
OEC	Observed Environmental Concentration
OECD	Organisation for Economic Co-operation and Development
PCB	Polychlorinated Biphenyl
PC*x*	Protective Concentration to x percentage of species. For example, PC95 represents a concentration that is theoretically protective of 95% of species. It is the converse of HC5, a concentration that is theoretically hazardous to 5% of species.
PERA	Probabilistic Ecological Risk Assessment
ppt	parts per thousand
QSAR	Quantitative Structure Activity Relationship
RPD	Relative Percent Difference
SE	Standard Error
SSD	Species Sensitivity Distribution

SVD	Springvale Drain
SVT	Springvale Tributary
TIE	Toxicity Identification Evaluation
TLM	Target Lipid Model
TTM	Total Toxicity of the Mixture
TU	Toxic Unit
TV	Trigger Value
USEPA	United States Environmental Protection Agency
VCH	Volatile Chlorinated Hydrocarbon
WET	Whole Effluent Toxicity
WQG	Water Quality Guidelines

INTRODUCTION

Estuaries are often the receiving ecosystems for inorganic and organic contaminants from groundwater, mining, agriculture, industry, stormwater and urbanisation (Bickford *et al.* 1999; Birch and Taylor 1999). Groundwater contaminated with chlorinated solvents is common (USEPA 1990) and is frequently a source of contamination to estuaries (Burton *et al.* 2002; Zolezzi *et al.* 2005). Water quality guidelines (WQGs) provide concentrations of contaminants that are predicted to be 'safe' for aquatic and marine ecosystems and in Australia, these WQGs are provided in the ANZECC and ARMCANZ (2000) Guidelines for Fresh and Marine Water Quality. Guidelines are, however, typically provided only for individual contaminants, not for the complex mixtures commonly encountered in the environment. Ecological risk posed by contaminants in the environment is typically assessed through the ecological risk assessment (ERA) process, developed in America (Suter 1993), and adopted in Australia (NEPC 1999). Techniques for use in quantitative ERA of contamination, however, are still being developed and the lack of data for Australian species (Warne and Westbury 1999) limits the ability of risk assessors to accurately assess risk for complex mixtures of chemicals.

Groundwater contaminated with volatile chlorinated hydrocarbons (VCHs) from historical releases of industrial chemicals was identified in Botany, near Sydney (Australia). The VCHs were discharging via stormwater drains into Penrhyn Estuary, a small intertidal embayment of Botany Bay. The estuary contains intertidal mudflats, seagrass beds, saltmarsh and mangrove vegetation and is a habitat for a variety of benthic and avian species, including migratory seabirds. The VCHs identified in the estuary act under the narcotic pathway (Carey *et al.* 1998), disrupting cellular functions through interference with membrane activities (Abernathy *et al.* 1988). Given the groundwater flow toward Penrhyn Estuary and Botany Bay, an assessment of the risk posed by VCHs to the aquatic receptors was warranted.

The current thesis is presented as a series of seven papers, all of which are at some stage in the process of journal publication.

The first paper describes a screening level ecological hazard assessment, undertaken to assess the hazard posed by the discharge of groundwater containing a complex mixture of VCHs into Penrhyn Estuary. ANZECC and ARMCANZ (2000) trigger values (TVs) were available for only 9 of the 14 VCHs identified in the surface water in the estuary. The objective of this part of the project was to derive TVs for the remaining 5 VCHs, for which existing TVs were not available and to assess the hazard posed by the VCHs to aquatic organisms in the estuary. The paper assessed the hazard at 2 locations in the source area and 7 locations in the estuary at high and low tides individually, and across both high and low tides, collectively. A number of limitations were identified in the screening level hazard assessment, and these were addressed in subsequent studies.

The second paper describes a methodology for undertaking toxicity testing in sealed containers to prevent loss of volatile contaminants, which would potentially underestimate toxicity. The methodology was evaluated for 6 indigenous marine test organisms including: oyster (*Saccostrea commercialis*) and sea urchin larvae (*Heliocidaris tuberculata*); a benthic alga (*Nitzschia closterium*); an amphipod (*Allorchestes compressa*); a larval fish (*Macquaria novemaculeata*); and a polychaete worm (*Diopatra dentata*). The study evaluated the suitability of these test vessels for use with the 6 organisms. The study also evaluated VCH loss in vials for small organisms (*H.tuberculata*, *S.commercialis* and *N.closterium*) and VCH loss from jars for larger organisms (*M.novemaculeata*, *A.compressa* and *D.dentata*).

The third paper describes direct toxicity assessment (DTA) and derivation of a site-specific guideline for the complex mixture of VCHs in groundwater. The DTA evaluated the toxicity of the groundwater to 5 marine test organisms. A site-specific species sensitivity distribution (SSDs) and 95% TV were derived for application to the receiving ecosystem of Penrhyn Estuary. Additional SSDs were derived to evaluate the effect of choice of distribution and manipulation of input data to the derivation of TVs.

The fourth paper presents a quantitative probabilistic ERA for the discharge of groundwater to Penrhyn Estuary. Risk was quantified using site-specific probability distributions for both toxicity and exposure assessments. Joint probability curves (JPCs) were derived for the 2 source areas and each of the 4 areas identified within the estuary and for each of high and low tides, individually and when both high and low tides were assessed together. Two toxicity scenarios were also evaluated to assess potential and likely adverse ecological effects to the estuary. Ecological risk was assessed by 2 measures, i.e. a quantified value of risk and the shape of the JPC.

The fifth paper evaluated the sensitivity of the test organisms used in the derivation of the site-specific guideline and the additivity of the toxicity of the complex mixture of VCHs in groundwater. As methods for direct measurement of VCHs in tissues of micro-organisms are not available, the evaluation was undertaken using predicted internal residues and narcotic toxicity associated with the critical body residue methodology.

The sixth paper evaluated the protectiveness of the existing ANZECC and ARMCANZ (2000) TVs for 2 of the VCHs identified in the groundwater mixture: chloroform and 1,2-dichloroethane. Toxicity testing was undertaken using seawater spiked with each of these contaminants. The study assessed whether the existing low reliability TVs provided in ANZECC and ARMCANZ (2000) are protective of indigenous marine organisms. Data generated in the study were used to derive new moderate reliability TVs for chloroform and 1,2-dichloroethane in accordance with the methodology provided in the ANZECC and ARMCANZ (2000) guidelines.

Following identification of potential risk to ecological receptors, a groundwater remediation system was commissioned in 2006 to prevent discharge of groundwater and greater contamination of the estuary. The seventh paper assesses the ecological risk following the implementation of groundwater remediation and reduction of contaminant load to the estuary. This paper quantifies the reduction in ecological risk and evaluates the

success of the remediation strategy using the measurement of ecological risk. Revisiting ecological risk assessments is vital to the success of ERA and central to the ERA framework and is seldom undertaken.

STATUS OF PAPERS

Paper 1: Published, *Australasian Journal of Ecotoxicology*, vol. 13, pp 33-42, 2007.

Paper 2: Published, *Bulletin of Environmental Contamination and Toxicology* vol. 82, pp 743-748, 2009.

Paper 3: Published, *Integrated Environmental Assessment and Management*, vol 5, no. 2, pp 338-348, 2009.

Paper 4: Accepted, *Environmental Toxicology and Chemistry*

Paper 5: Not yet submitted

Paper 6: Accepted, *Australasian Journal of Ecotoxicology*

Paper 7: Not yet submitted

PAPER 1

DERIVING TRIGGER VALUES FOR, AND ASSESSING HAZARD POSED BY, VOLATILE CHLORINATED HYDROCARBONS IN A SYDNEY ESTUARY

ABSTRACT

The hazard of adverse effects to aquatic organisms arising from groundwater contaminated with a complex mixture of volatile chlorinated hydrocarbons (VCHs) discharging to Penrhyn Estuary, Sydney, Australia was determined using the hazard quotient (HQ) method. The hazard posed by 14 VCHs acting individually and collectively as a mixture was assessed. The hazard was determined using measured aqueous concentrations of the VCHs (at high and low tide and an average of the two) and the corresponding trigger values from the Australian and New Zealand water quality guidelines. *Low reliability* trigger values were derived in this study for five VCHs, in accordance with the methodology in Australian and New Zealand water quality guidelines. High ecological hazard was posed by some individual VCHs at sites close to the contaminant source in the upper and inner estuary, while the corresponding hazard in the outer estuary was low. The hazard was always greater at low tide than high tide in the inner and outer estuary, presumably due to the inflow of tidal water and subsequent dilution of contaminants. The chemicals that posed the greatest hazard were 1,2-dichloroethane, tetrachloroethene and vinyl chloride. Six of the nine sites received a high hazard for at least one VCH. Assessing the hazard posed by the mixture always increased the HQ values compared to that for individual chemicals but it did not increase the number of sites which received a high hazard (i.e. 6 of 9 sites). The hazard assessment indicates that more detailed assessment in the form of a probabilistic risk assessment and direct toxicity assessment is warranted. Limitations identified in this hazard assessment include the use of *low reliability* trigger values for individual chemicals, additivity of toxicants and highly variable (spatially and temporally) concentrations.

INTRODUCTION

Estuaries are often the receiving ecosystems for inorganic and organic contaminants (Brown and Ferris 2004; Pankow *et al.* 2006) from industry (Bervoets *et al.* 1996; Jin *et al.* 1999), mining (Twining and Cameron 1997; Lakatos *et al.* 2003), urbanisation (Bickford *et al.* 1999; Birch and Taylor 1999) and agriculture (Poletika *et al.* 2002; Villa *et al.* 2003). Stormwater in Sydney catchments has been identified as a source of a wide range of contaminants to estuaries, including industrial chemicals, sewage overflows, gardening products and vehicle exhaust particulate matter and metals (Birch *et al.* 1996; Bickford *et al.* 1999; Birch and Taylor 1999). Contaminated groundwater may also be a source of contaminants to aquatic ecosystems (Burton *et al.* 2002; Zolezzi *et al.* 2005) and groundwater contamination by volatile chlorinated hydrocarbons (VCHs) and industrial solvents is common internationally (USEPA 1990; Pohl *et al.* 2003; Zolezzi *et al.* 2005).

Penrhyn Estuary is a small artificially created tidal embayment on the northern shoreline of Botany Bay, Sydney, Australia. It was originally devoid of vegetation and wildlife when it was created in the late 1970s using sandy dredge spoil from the adjacent port development. Today, however, it supports a variety of flora species, including mangroves, saltmarsh species and dune vegetation and also attracts wading shorebirds which forage on the mudflats at low tide. Previous investigations have identified shallow groundwater that flows into two drains – Springvale and Floodvale – and then into Penrhyn Estuary, is contaminated by 14 VCHs (Table 1) (AGEE and Woodward-Clyde 1990; Woodward-Clyde 1996; URS 2004b). Contamination of surface water in the estuary with VCHs has been continuous since at least 1990. To date, assessment of the environmental hazard to aquatic organisms posed by the contamination has not been undertaken.

Published hazard assessments of the potential impact of VCHs are limited. A regional scale hazard assessment for VCHs in surface waters has been conducted for Europe (Garny *et al.* 1998). No published studies have assessed the hazard posed by VCHs in surface water in Australia and at present, there is an absence of toxicity data for indigenous Australian species (Warne and Westbury 1999).

In contrast, the potential ecological risks posed by non-volatile chlorinated hydrocarbons (e.g. dioxins and polychlorinated biphenyls - PCBs) have been extensively investigated (McCarty and Mackay 1993b; Carey *et al.* 1998) over the past two decades. However, findings from these studies are unlikely to be relevant for VCHs as the two groups of chemicals have markedly different physicochemical properties and environmental behaviours.

VCHs are characterised by low boiling points, high vapour pressures, generally high aqueous solubility and low octanol-water partition coefficients (K_{ow}). As a result they have a low potential: to bioaccumulate (Carey *et al.* 1998); and to bind to organic carbon, sediment, or suspended particulate matter. The predicted equilibrium distribution of VCHs in the environment is approximately 99% in the atmosphere and 1% in water (Carey *et al.* 1998). In contrast, non-volatile chlorinated hydrocarbons tend to have low aqueous solubility and high K_{ow} values and thus bind extensively to sediment and particulate matter and strongly bioaccumulate. VCHs exert toxicity by the non-polar narcotic mode of action (Carey *et al.* 1998).

Therefore, the primary objective of this paper was to determine the hazard posed by the 14 VCHs when acting individually and collectively as a mixture to aquatic organisms in Penrhyn Estuary. The secondary objective was to identify potential limitations of the hazard assessment in order to improve subsequent, higher-tier risk assessments, should they be necessary.

METHODOLOGY

EXPOSURE ASSESSMENT

Penrhyn Estuary is located on the northern shore of Botany Bay, 10 km to the south of the Sydney central business district. The 10 ha study area included approximately 500 m and 300 m of Springvale and Floodvale drains respectively and the inner and outer parts of the estuary (Figure 1).

Nine sites were selected and established to characterise various zones within the study area (Table 2, Figure 1). Sites 1 and 2 were in source areas upstream of Penrhyn Estuary in the Springvale and Floodvale Drains, respectively. Sites 1 and 2 are located within a stormwater drainage system and are not considered to be part of the estuarine ecosystem, but have been included for comparative purposes and source characterisation. Sites 3, 4 and 5 were in the upper estuary; Sites 6, 7 and 9 were in the inner estuary; and Site 8 was in the outer estuary.

Sampling of estuarine water from the sites and subsequent analysis for VCHs was undertaken over a one-year period (in 2004 and 2005) in two monitoring programs. In the first program, five rounds of samples were collected every three months from Sites 1, 2, 4, 5, 6, 8 & 9 to assess variability in concentrations of VCHs in the estuary over one year (URS 2004b). In the second program nine rounds of samples were collected over one month from Sites 1 and 3 to 8 to assess short-term variability (URS 2005). As the estuary is tidal, samples were collected at both high and low tides at Sites 3 to 8 irrespective of the sampling program. One sampling round was common to both programs, hence the maximum number of rounds at any given site was 13 (Table 2).

Three exposure scenarios were assessed at each site; Scenario 1 - the mean high tide aqueous concentration, Scenario 2 - the mean aqueous concentration (i.e. the mean of the high and low tide concentration data), and Scenario 3 - the mean low tide aqueous concentration.

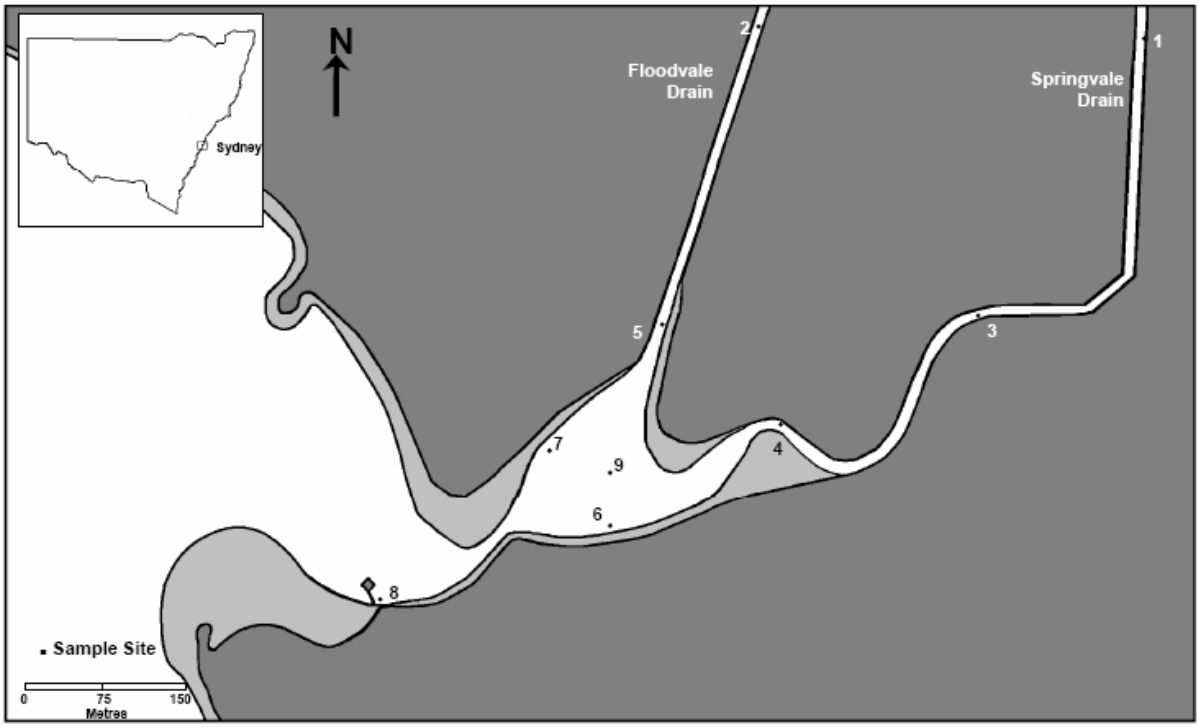


Figure 1. Sample Sites in Penrhyn Estuary

Samples were collected in 40 mL glass vials with airtight Teflon™ lined lids with zero headspace. They were preserved in the field with hydrochloric acid and immediately stored at less than 4°C. Samples were extracted using purge and trap methodology (USEPA 5030B) and analysed by Gas Chromatography Mass Spectrometry (GC/MS) utilising a modification of the USEPA Method 8260B for volatile organic compounds (USEPA 1996c). The limit of reporting was 1 µg/L for all analytes with the exception of vinyl chloride (10 µg/L). Quality control evaluations were undertaken on each of the sample batches. No analytes were detected in the method blanks and recoveries for laboratory control samples and matrix spikes were between 80 to 120%, and within the accepted criteria. Differences between primary and duplicate samples were generally less than 25%. This difference is typical of the variability observed between duplicate samples for these contaminants at this laboratory and is considered acceptable (URS, 2004; 2005).

EFFECTS ASSESSMENT

The effects assessment component is commonly undertaken using either toxicity data or water quality guidelines (WQGs) (USEPA 1998; NEPC 1999). There is a lack of toxicity data for the 14 VCHs to indigenous Australian species (Warne and Westbury 1999), therefore, water quality guidelines were used. In Australia, the numerical limits for chemicals are termed trigger values (TVs) as if these are exceeded, further action is triggered. The documents that the TVs are collated in (ANZECC and ARMCANZ 2000) are referred to as the WQGs (Warne 2001). Trigger values are provided in the WQGs (ANZECC and ARMCANZ 2000) that aim to protect different percentages of species (i.e. 99%, 95%, 90% and 80%). In this study, we used the TVs that aim to protect 95% of species (i.e. PC95) (ANZECC and ARMCANZ 2000) and thus theoretically permit only 5% of species to suffer sub-lethal chronic toxic effects. These TVs (PC95) are therefore analogous to the hazardous concentrations to 5% of species (HC5) commonly used in Europe. TVs were available for nine of the 14 chemicals (ANZECC and ARMCANZ, 2000) (Table 1) and TVs from other jurisdictions could not be found for the remaining five. Therefore, TVs for the remaining five contaminants, namely 1,1,1,2-tetrachoroethane, 1,1-dichloroethane, 1,1-dichloroethene, *cis* 1,2-dichloroethene and *trans* 1,2-dichloroethene, were derived using the quantitative structure-activity relationship (QSAR) methodology and Burrlioz software (Campbell *et al.*, 2000) to derive species sensitivity distributions (Burr Type III distributions) for the

contaminants and TVs in the manner presented in ANZECC and ARMCANZ (2000) for other VCHs. The resulting PC95 values were deemed to be *low reliability* TVs as recommended in the WQGs (ANZECC and ARMCANZ, 2000).

HAZARD ASSESSMENT

Hazard assessments are often undertaken using the Hazard Quotient (HQ) approach (Urban and Cook 1986; NEPC 1999). The HQ approach uses point estimates of exposure and toxicity. In this study, the HQ was calculated for chemical 'i' by dividing the observed environmental concentration (OEC_i) by its corresponding TV_i:

$$HQ_i = OEC_i / TV_i \quad (1)$$

The OEC_i is equivalent to the mean water concentration at each site for each scenario. The hazard posed by each of the 14 VCHs was determined using three different OEC_i values – corresponding to the three scenarios described in the previous section (Table 3).

A HQ value of less than 0.5 indicated that the TV had not been exceeded and the chemical posed a low hazard. A HQ value of between 0.5 and 1.0 indicated that the TV had not been exceeded and a moderate hazard existed. A HQ value of greater than 1.0 indicated that a high hazard existed, the TV had been exceeded, and thus 95% of species would not be protected. When the HQ is greater than one, further hazard/risk assessment is required. These cut-offs applied to all three scenarios.

The total toxicity of the mixture of the 14 VCHs was determined for each exposure scenario in order to provide a more environmentally realistic assessment of the hazard posed (Table 4). The HQ approach presented in equation 1 required modification in order to be applicable to contaminant mixtures. Research by various groups (Broderius and Kahl, 1985; Hermens *et al.*, 1985; Altenburger *et al.*, 2000) has showed that mixtures of contaminants with a common mode of action, as is the case here, tend to exert toxicity that is consistent with concentration addition, whereas for compounds that exert toxicity via different modes of action, the toxicity is generally consistent with response addition. The Australian and New Zealand WQGs

(ANZECC and ARMCANZ, 2000) state that compounds that exert their toxicity by a narcotic mode of action or are likely to elicit additive effects when present in a mixture should have their combined toxicity determined using the formula:

$$TTM = \Sigma (C_i / TV_i) \quad (2)$$

where TTM is the predicted total toxicity of the mixture, C_i is the mean concentration of the 'i'th component of the mixture and TV_i is the corresponding trigger value. A mixture with a TTM of less than 0.5 indicates a low hazard, a TTM between 0.5 and 1.0 indicates a moderate hazard, while a TTM greater than 1.0 indicates a high hazard, triggering further investigation.

The total toxicity of the mixture (TTM), akin to the toxic unit (TU) approach, accounts for potential additive effects of VCHs and is equivalent to the addition of HQs for individual contaminants. It is recommended in the Australian and New Zealand WQGs (ANZECC and ARMCANZ, 2000) that the TTM method only be used on mixtures containing no more than five toxicants. For mixtures with more than five components it is recommended that the toxicity of the mixture be measured using direct toxicity assessment (DTA) methods. As we were conducting a screening level hazard assessment, it was decided to apply the TTM method to the mixture of 14 VCHs (Table 4) even though this is not strictly in accordance with the Australian and New Zealand WQGs (ANZECC and ARMCANZ, 2000) recommendations.

RESULTS AND DISCUSSION

EXPOSURE ASSESSMENT

For Springvale Drain, the mean concentration of total VCHs decreased by more than one order of magnitude between the source area (Site 1; 22 055 µg/L) and the inner estuary (Site 6; 884 µg/L) and decreased by a further order of magnitude between the inner estuary and the outer estuary (Site 8; 85 µg/L) (Table 1). Maximum mean concentrations for VCHs were reported at Site 1 in the source area. Minimum concentrations of VCHs were reported at Sites 8 and 9, furthest from the source of contamination.

Concentrations decreased by half an order of magnitude from 1425 µg/L to 400 µg/L between the source area and the discharge point of Floodvale Drain. Mean concentrations of total VCHs in Springvale Drain (22 055 µg/L) were approximately one order of magnitude higher than those in Floodvale Drain (1425 µg/L) (Table 1).

Approximately 70% of total VCHs in samples consisted of 1,2-dichloroethane. The proportion of vinyl chloride generally increased with distance down the drain flowpath of Springvale Drain and into the inner estuary from Site 1 to Site 6 increasing from approximately 8% to 19% as a proportion of total VCHs. Vinyl chloride formation results from breakdown of other VCHs (Lampron *et al.* 2001; Jones *et al.* 2004).

Concentrations of total VCHs were greater at low tide than high tide and the ratio of concentrations of total VCHs at low to high tide varied from a minimum of 2 (Site 4) to a maximum of 17.5 (Site 6) (Table 2). The smallest differences between high and low tide concentrations were at Sites 3, 4 and 5, which were closest to the discharge from the drains and therefore, had the most stable VCH concentrations.

The results indicate that the concentrations of VCHs in estuarine waters were highly variable, both spatially and temporally over the sampling period of one year. The ratio of maximum to

Table 1. Mean concentrations (µg/L) and trigger values of individual volatile chlorinated hydrocarbons at sample sites.

Analyte	ANZECC & ARMCANZ (2000) trigger values	Location								
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Carbon tetrachloride	240	346	9.10	34.7	9.75	2.23	6.85	2.06	1.27	0.80
Chloroform	370	496	27.2	98.9	41.6	11.1	21.8	6.17	2.71	2.59
1,1,1,2-Tetrachloroethane	*380*	7.38	1.70	1.40	0.61	0.50	0.73	0.50	0.50	0.50
1,1,2,2-Tetrachloroethane	400	29.7	3.80	12.7	4.33	1.17	2.92	0.72	0.73	0.56
1,1,1-Trichloroethane	270	7.38	1.30	1.85	0.61	0.50	0.73	0.50	0.50	0.50
1,1,2-Trichloroethane	1,900	114	11.8	23.6	8.39	3.75	6.44	1.47	0.98	1.00
1,1-Dichloroethane	*1,450*	48.7	14.2	11.9	5.64	4.17	2.98	1.14	0.73	0.78
1,2-Dichloroethane	1,900	17 573	1 156	2 741	746	249	583	152	53.2	56.1
Tetrachloroethene	70	690	6.20	73.5	27.9	1.60	14.4	4.19	2.06	1.13
Trichloroethene	330	490	33.0	124	49. 9	9.81	27.2	6.86	3.56	3.16
1,1-Dichloroethene	*3,900*	116	5.10	8.21	4.61	1.85	2.29	0.92	0.65	0.56
cis-1,2-Dichloroethene	*1,250*	454	42.4	238	93. 6	16.9	45.7	11.3	6.12	5.73
trans-1,2-Dichloroethene	*770*	23.5	11.4	19.5	9.75	3.56	4.13	1.53	0.87	1.02
Vinyl chloride	100	1 660	102	439	262	94.3	164	41.7	11.2	12.6
Total Chlorinated Hydrocarbons	Not Available	**22 055**	**1 425**	**3 828**	**1 266**	**400**	**884**	**231**	**85**	**87**

Trigger values shown in italics were derived in the present study using the same method as that used to derive trigger values for volatile chlorinated hydrocarbons in the Australian and New Zealand water quality guidelines (ANZECC and ARMCANZ, 2000).

Table 2. Sample descriptions, mean concentrations (µg/L) and standard deviations (St Dev) of total volatile chlorinated hydrocarbons and ratios of high and low tide concentrations and minimum to maximum concentrations.

	Location	Tidal	No. of Rounds	Concentration of Total VCHs								Ratio	
				Mean	St Dev	Mean High Tide	St Dev	Mean Low Tide	St Dev	Min	Max	Low /High Tide	Max/ Min
Site 1	Springvale Drain in an upstream source area	No	13	22 055	18 883	--	--	--	--	2 160	65 264	--	30
Site 2	Floodvale Drain in an upstream source area	No	5	1 425	689	--	--	--	--	482	2 427	--	5
Site 3	Penrhyn Estuary at the discharge point of Springvale Drain	Yes	9	3 828	5 425	2 089	2 118	5 566	7 171	107	26 574	2.7	250
Site 4	Upper Penrhyn Estuary	Yes	13	1 266	1 562	832	1 599	1 699	1 484	32	5 490	2.0	174
Site 5	Penrhyn Estuary at the discharge point of Floodvale Drain	Yes	13	400	314	195	136	606	310	41	1 183	3.1	29
Site 6	Old boat ramp in inner estuary on the southern shore	Yes	13	884	1 631	95.8	130	1 671	2 045	11	5 299	17.5	505
Site 7	Located on the northern shoreline opposite Station 6	Yes	9	231	327	75.3	83	386	407	7	1 342	5.1	192
Site 8	New boat ramp in the outer estuary on the southern shore	Yes	13	85	162	21.0	24	149	213	7	710	7.1	101
Site 9	Pooled data from 6 locations in the centre of the inner estuary	Yes	5	87	--	--	--	--	--	18	295	--	17

-- no data available

minimum concentration of VCHs at each site varied from a minimum of 5 (Site 2) to a maximum of 505 (Site 6; Table 2). The mean ratio between maximum and minimum concentrations at each site was 145 over one year. Previous studies undertaken in the estuary indicated that concentrations of VCHs in estuarine water were affected by rainfall, concentrations discharged into stormwater drains, tidal height (spring/neap) and tidal phase (diurnal inequity)(URS 2005). Although the use of mean concentrations may underestimate the hazard compared to using maximum concentrations, this approach is considered appropriate to characterise the chronic hazard arising from prolonged exposure (Muschal and Warne, (2003b) and supported by regulatory guidance (ANZECC and ARMCANZ 2000).

Effects Assessment

New trigger values derived for 1,1,1,2-tetrachoroethane, 1,1-dichloroethane, 1,1-dichloroethene, *cis* 1,2-dichloroethene and *trans* 1,2-dichloroethene varied from 380 µg/L to 3900 µg/L (Table 1). The range of TVs was similar to that of existing TVs for VCHs, which varied from 70 µg/L to 1900 µg/L.

Hazard Assessment - Individual Contaminants

Over all scenarios the HQ values ranged from 0.00 to 16.6. If the single highest HQ value for each combination of chemical and site was examined (i.e. HQ values for Site 9 for Scenario 1, Sites 1 & 2 from Scenario 2, and Sites 3 to 8 for Scenario 3), then a total of 126 HQ values are obtained. Of these 13 had a high hazard (~10%), 4 had a moderate hazard (~3%) and 109 (~87%) had a low hazard. Thus, overall the vast majority of site and chemical combinations pose a low hazard. However, 6 of the 14 chemicals pose a high hazard to at least one site and six of the nine sites are at a high hazard from at least one of the chemicals.

Of the chemicals posing a high hazard, vinyl chloride poses a high hazard to the most sites (i.e. Sites 1 to 6). Tetrachloroethene and 1,2-dichloroethane both pose a high hazard to two sites, while carbon tetrachloride, chloroform and trichloroethene only posed a high hazard to one site (i.e. Site 1). This hazard is driven by the initial high concentrations of these contaminants at the source area, which generally decrease along the flowpath.

In Scenario 1 (mean high tide concentrations) there were a total of 98 HQ values, with 2 (2%) having a high hazard, 2 (2%) having moderate hazard and 94 (96%) having low hazard. In Scenario 2 (mean of high and low tide concentrations) there were 112 HQ values. Of these 12 (11%) were of high hazard, 2 (2%) had moderate hazard and 98 (87%) had low hazard. For Scenario 3 (low tide concentrations) there was a total of 84 HQ values of which 6 (7%) had a high hazard, 3 (4%) had a moderate hazard and 75 (89%) had a low hazard.

The three scenarios contain HQ values for different sites (as described in the methods section) which have different environmental concentrations due to their distance from the contaminant source (refer to Figure 1). Therefore, comparisons across all exposure scenarios can only be made by comparing the HQ values from the sites common to all three scenarios (i.e. Sites 3 to 8, Table 3). This comparison indicates that both the magnitude and number of high hazard HQ values increased as the tide decreased. This is most probably due to the contaminated groundwater being a larger proportion of the water at any given site at low tide, while at high tide, the proportion of contaminated groundwater would be decreased by the influx of marine water into the tidal reaches of the estuary (i.e. at Sites 4 to 9).

Hazard quotients and the potential ecological hazard decreased in the following order source area, upper estuary, inner estuary, and outer estuary (Table 3). This trend is consistent with loss of contaminants from the waters through volatilisation and increased dilution with water from Botany Bay.

The HQ scale is non-linear. Thus, it is invalid to conclude that a HQ value of 10 is 5 times worse than a value of 2, nor can hazard be quantitatively compared between different contaminants (Tannenbaum *et al.* 2003). This arises when HQs are calculated by dividing the lowest toxicity value by the highest environmental concentration because concentration-response curves or probability are not included (Sorenson *et al.* 2004). In the present study HQ was calculated by dividing the TV by the environmental concentrations of the chemical in three different scenarios. The lack of linearity in this present study arises because the species sensitivity distribution for chemicals is not linear but usually sigmoidal. Using the HQ approach, the interpretation of potential ecological hazard was restricted to the defined

classifications of low, moderate and high hazard, with high hazard indicating that 95% of species may not be protected.

Assessment of surface water concentrations of the VCHs showed that these were highly variable, both spatially and temporally (Table 2). For example, the difference between the smallest and largest concentration of total VCHs across all sites was approximately 9300. The variation within individual sites across time ranged from 5 fold to 500 fold. Therefore, the exposure of organisms to VCHs and the potential ecological hazard is also likely to be highly variable. This highly variable exposure may not be adequately quantified by the use of mean concentrations of VCHs to represent exposure. But as the measured concentrations are from grab samples we have no idea of how long the measured concentrations persisted for. Thus, using the average concentration in the HQ calculations provides an estimate of the average concentration the organisms were exposed to and the WQGs are for chronic exposure. To account for variability in the exposure of organisms, probabilistic techniques should be used, and this will be done in subsequent work.

Table 3. Calculated hazard quotients for the nine volatile chlorinated hydrocarbons (VCHs) measured at each sampling site under the three exposure scenarios

Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Scenario 1 - Hazard quotients for mean VCH concentrations at high tide									
Carbon tetrachloride	--	--	0.09	0.03	0.01	0.00	0.00	0.00	0.00
Chloroform	--	--	0.19	0.07	0.02	0.01	0.01	0.00	0.01
1,1,2,2-Tetrachloroethane	--	--	0.02	0.01	0.00	0.00	0.00	0.00	0.00
1,1,1-Trichloroethane	--	--	0.01	0.00	0.00	0.00	0.00	0.00	0.00
1,1,2-Trichloroethane	--	--	0.01	0.00	0.00	0.00	0.00	0.00	0.00
1,2-Dichloroethane	--	--	*0.68*	0.27	0.07	0.03	0.02	0.00	0.03
Tetrachloroethene	--	--	*0.76*	0.32	0.02	0.03	0.02	0.01	0.02
Trichloroethene	--	--	0.28	0.09	0.02	0.01	0.01	0.00	0.01
Vinyl chloride	--	--	**3.24**	**1.66**	0.38	0.17	0.20	0.06	0.13
1.1.1.2-Tetrachloroethane	--	--	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1.1-Dichloroethane	--	--	0.01	0.00	0.00	0.00	0.00	0.00	0.00
1.1-Dichloroethene	--	--	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cis-1.2-Dichloroethene	--	--	0.14	0.04	0.01	0.01	0.00	0.00	0.00
trans-1.2-Dichloroethene	--	--	0.02	0.01	0.00	0.00	0.00	0.00	0.00

Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Scenario 2 - Hazard quotients for mean VCH concentrations at both high and low tides									
Carbon tetrachloride	**1.44**	0.04	0.14	0.04	0.01	0.03	0.01	0.01	--
Chloroform	**1.34**	0.07	0.27	0.11	0.03	0.06	0.02	0.01	--
1,1,2,2-Tetrachloroethane	0.07	0.01	0.03	0.01	0.00	0.01	0.00	0.00	--
1,1,1-Trichloroethane	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00	--
1,1,2-Trichloroethane	0.06	0.01	0.01	0.00	0.00	0.00	0.00	0.00	--
1,2-Dichloroethane	**9.25**	*0.61*	**1.44**	0.39	0.13	0.31	0.08	0.03	--
Tetrachloroethene	**9.85**	0.09	**1.05**	0.40	0.02	0.21	0.06	0.03	--
Trichloroethene	**1.49**	0.10	0.37	0.15	0.03	0.08	0.02	0.01	--
Vinyl chloride	**16.60**	**1.02**	**4.39**	**2.62**	*0.94*	**1.64**	0.42	0.11	--
1.1.1.2-Tetrachloroethane	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--
1.1-Dichloroethane	0.03	0.01	0.01	0.00	0.00	0.00	0.00	0.00	--
1.1-Dichloroethene	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	--
cis-1.2-Dichloroethene	0.36	0.03	0.19	0.07	0.01	0.04	0.01	0.00	--
trans-1.2-Dichloroethene	0.03	0.01	0.03	0.01	0.00	0.01	0.00	0.00	--

Site	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Scenario 3 - Hazard quotients for mean VCH concentrations at low tide									
Carbon tetrachloride	--	--	0.20	0.05	0.01	0.05	0.01	0.01	--
Chloroform	--	--	0.35	0.16	0.05	0.11	0.03	0.01	--
1,1,2,2-Tetrachloroethane	--	--	0.04	0.02	0.00	0.01	0.00	0.00	--
1,1,1-Trichloroethane	--	--	0.01	0.00	0.00	0.00	0.00	0.00	--
1,1,2-Trichloroethane	--	--	0.02	0.01	0.00	0.01	0.00	0.00	--
1,2-Dichloroethane	--	--	**2.20**	*0.52*	0.20	*0.58*	0.14	0.05	--
Tetrachloroethene	--	--	**1.34**	0.46	0.02	0.39	0.10	0.05	--
Trichloroethene	--	--	0.47	0.21	0.04	0.15	0.03	0.02	--
Vinyl chloride	--	--	**5.53**	**3.59**	**1.51**	**3.12**	*0.63*	0.17	--
1.1.1.2-Tetrachloroethane	--	--	0.01	0.00	0.00	0.00	0.00	0.00	--
1.1-Dichloroethane	--	--	0.01	0.01	0.00	0.00	0.00	0.00	--
1.1-Dichloroethene	--	--	0.00	0.00	0.00	0.00	0.00	0.00	--
cis-1.2-Dichloroethene	--	--	0.24	0.11	0.02	0.07	0.01	0.01	--
trans-1.2-Dichloroethene	--	--	0.03	0.02	0.01	0.01	0.00	0.00	--

normal numbers - denote a low hazard (HQ of less than 0.5)
italicised numbers - denote a moderate hazard (HQ between 0.5 and 1.0) and
bold numbers - denote a high hazard (HQ of greater than 1.0)
-- no data available

Table 4. Total toxicity of the volatile chlorinated hydrocarbon mixture (TTM) for sites 1 to 9 under three exposure scenarios.

Scenario	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
Scenario 1 - Mean concentrations at high tide	--	--	**5.44**	**2.50**	*0.52*	0.26	0.27	0.08	0.20
Scenario 2 - Mean concentrations at high and low tides	**40.61**	**2.01**	**7.95**	**3.83**	**1.20**	**2.38**	*0.62*	0.20	--
Scenario 3 - Mean concentrations at low tide	--	--	**10.45**	**5.16**	**1.87**	**4.51**	*0.97*	0.33	--

normal numbers - denote a low hazard (TTM of less than 0.5)
italicised numbers - denote a moderate hazard (TTM between 0.5 and 1.0) and
bold numbers - denote a high hazard (TTM of greater than 1.0)
-- no data available

HAZARD ASSESSMENT - CONTAMINANT MIXTURE

The HQ values for mixtures ranged from 0.08 to 40. These are considerably higher than the HQ values obtained from the hazard assessment for individual chemicals (compare Tables 3 and 4). This is expected as the TTM sums the toxic effects of each individual chemical.

If you collate the single highest HQ value for each site there is a total of nine HQ values. Of these six posed a high hazard (~67%), one posed a moderate hazard (~11%) and two posed a low hazard (~22%). Thus, the vast majority of sites would be exposed to a high hazard from the contaminant mixture. The same number of sites was exposed to high hazard in the hazard assessment conducted for individual chemicals and the mixtures. Despite this finding, it is necessary to assess the toxicity of the mixture of VCHs mixture in order to adequately assess the potential hazard posed to the sites in Penrhyn Estuary.

The trends of the key results from the mixture hazard assessment are the same as those for individual chemicals. The magnitude of the HQ values and the number of high hazard HQ values increased with decreasing tide. The HQ values decreased in size with increasing distance from the contaminant source. Springvale Drain poses a greater hazard than Floodvale Drain.

LIMITATIONS OF THE HAZARD ASSESSMENT

There are limitations to the hazard assessment conducted in this study and the potential effects that these had warrant discussion.

Firstly, the trigger values are classed as *low reliability* (ANZECC and ARMCANZ, 2000), meaning that there is lower confidence that the TVs will provide the stated level of protection (i.e. 95% of species) as in this case, they are based on predicted rather than experimentally derived toxicity data (Warne 2001). The predicted data are acute no observed effect concentration (NOEC) data generated by quantitative structure activity relationships (QSARs) for non-polar narcotic chemicals. ANZECC and ARMCANZ

(2000) recommends that *low reliability* values not be used as default guidelines, noting however, that it is reasonable to use them in a risk-based decision scheme to determine if conditions at a site increase or decrease risk. As there was a paucity of available toxicity data, *low reliability* TVs were used in this hazard assessment. It is recommended that empirical research be undertaken to evaluate the validity of the *low reliability* TVs for VCHs.

The second limitation of the TVs is that they are based on toxicity data for freshwater species as there was no toxicity data for estuarine or marine organisms. In such cases the Australian and New Zealand WQGs (ANZECC and ARMCANZ, 2000) recommends that the fresh water TVs be adopted to marine waters. There is the inherent assumption made in doing this, that the sensitivity of freshwater and marine organisms is the same. This assumption has still not been resolved with some supporting it (van Wezel and Jonker 1998) and others showing that it is not always valid (Leung *et al.* 2001). Despite the uncertainty regarding the underlying assumption we adopted these TVs as they are the Australian WQGs and there is no alternative as insufficient data on estuarine or marine species are available.

Given the number of high hazard results obtained it was earlier recommended that probabilistic risk assessment be conducted on Penrhyn Estuary. In order for this to be rigorous it would be highly advantageous if toxicity testing using local estuarine or marine species was conducted and *moderate*- or *high reliability* TVs derived for all or at least some of the 14 VCHs measured at Penrhyn Estuary.

The VCHs assessed typically have low K_{ow} values, high water solubility, do not bioaccumulate and both uptake and depuration of these compounds is likely to be relatively rapid and metabolism has been shown for higher organisms (i.e. fish) (Carey *et al.* 1998). Despite this, the potential hazard of the metabolites of the VCHs was not assessed in the present study. It is not possible to know what affect the inclusion of metabolites and degradates in the hazard assessment would have. However, this limitation (not accounting for metabolites or depredates) applies to the vast majority of hazard and risk assessments due to a lack of knowledge of the metabolic breakdown of chemicals and a lack of toxicity data.

UNCERTAINTY IN ECOLOGICAL HAZARD ASSESSMENT

Sources of uncertainty in the present ERA include: extrapolation of laboratory toxicity data to the ecosystem; the use of the HQ approach; the highly variable exposure; the use of *low reliability* water quality guidelines for toxicity assessment; the presence of multiple potentially interacting contaminants; and the dynamic salinity of the receiving ecosystem.

CONCLUSIONS

Low reliability trigger values (TVs) were derived for five VCHs, namely 1,1,1,2-tetrachoroethane, 1,1-dichloroethane, 1,1-dichloroethene, *cis* 1,2-dichloroethene and *trans* 1,2-dichloroethene, for which water quality guidelines were not previously available. These new TVs ranged from 380 µg/L to 3900 µg/L. These TVs were used with the existing TVs for VCHs to screen the hazard posed by VCH contamination to Penrhyn Estuary.

The hazard assessment identified both high and moderate ecological hazards to aquatic organisms residing in the upper and inner estuary areas. Low ecological hazard was identified in the outer estuary. Ecological hazard was greater at low tide than at high tide throughout the estuary. Concentrations of VCHs were highly variable both spatially and temporally, resulting in spatially and temporally variable potential ecological hazard. Therefore, higher tiers of risk assessment should address the potential interaction of contaminants in the mixture through DTA and should address the variability in the exposure through the use of probabilistic techniques.

PAPER 2

EVALUATION OF A METHODOLOGY FOR TOXICITY TESTING OF VOLATILE CHLORINATED HYDROCARBONS ON MARINE ORGANISMS

ABSTRACT

This study evaluated the suitability of sealed containers for toxicity testing to prevent loss of volatile chlorinated hydrocarbons (VCHs) with a range of Australian marine organisms including: micro-algae (*Nitzschia closterium*); sea urchin (*Heliocidaris tuberculata*) and oyster (*Saccostrea commercialis*) larvae in 44 mL sealed vials and fish larvae (*Macquaria novemaculeata*); amphipods (*Allorchestes compressa*); and juvenile polychaetes (*Diopatra dentata*) in 1 L sealed jars. Vials prevented volatilisation of VCHs during testing. Jars were less effective, with average losses of 46%. Growth and development of algae, sea urchins and oysters in vials was acceptable, indicating suitability of the methodology. Jars were suitable for amphipods and polychaetes; however, further evaluation of the fish test is required.

INTRODUCTION

Penrhyn Estuary, in Sydney, Australia, receives groundwater contaminated with volatile chlorinated hydrocarbons (VCHs) and comparison of measured concentrations of VCHs in estuarine waters against the numerical limits (termed trigger values – TVs) for these chemicals in the Australian water quality guidelines (ANZECC and ARMCANZ 2000) indicated that VCHs posed an unacceptable hazard and that direct toxicity assessment (DTA) was warranted (Hunt *et al.* 2007). However, TVs for VCHs are classed as *low reliability* – meaning the amount and type of toxicity data on which they are based are not optimal. The Australian water quality guidelines (ANZECC and ARMCANZ 2000) identified generating additional toxicity data for chemicals with *low reliability* TVs as a key research priority. For both these reasons it is necessary to conduct toxicity tests where indigenous species are exposed to VCHs.

VCHs are characterised by high vapour pressures and Henry's Law Constants and are readily lost from open containers. As such, standard test protocols are not suitable, as volatilisation of VCHs would result in decreased exposure concentrations and underestimation of the toxicity. Recent work by Tsai and Chen (2007) indicated that toxicity testing for volatile narcotic contaminants undertaken in open containers underestimated toxicity to algae, when compared to testing in closed systems, by up two orders of magnitude, regardless of Henry's Law Constants. Although studies have been undertaken to develop test protocols for sealed test vessels, these have focussed on micro-algae (Galassi and Vighi 1981; Herman *et al.* 1990; Brack *et al.* 1998; Mayer *et al.* 2000; Chen and Lin 2005; Lin *et al.* 2005), with some assessment of the suitability of sealed test vessels for cladocerans (Rose *et al.* 1997). Limited assessment of the suitability of these methods has been undertaken for other test organisms. This study presents a methodology for determining the toxicity of VCHs in sealed vessels for six indigenous marine organisms including: a sea urchin (*Heliocidaris tuberculata*); an oyster (*Saccostrea commercialis*); a micro-alga (*Nitzschia closterium*); a fish

(*Macquaria novemaculeata*); an amphipod (*Allorchestes compressa*) and a polychaete (*Diopatra dentata*). Development of this methodology was done to support DTA of VCH contamination and evaluation of the appropriateness of the ANZECC and ARMCANZ (2000) trigger values for VCHs. Three contaminant treatments were evaluated; a complex mixture of 14 VCHs in groundwater from an industrial facility, which is the source of discharge to Penrhyn Estuary, and individual seawater samples spiked with 1,2-dichloroethane and chloroform.

To accurately attribute toxic effects to concentrations of a chemical in toxicity tests requires constant, known concentrations (Simpson et al. 2003). It is, however, still common practice to use nominal or measured initial concentrations of contaminants for calculation of toxicity even though it is known that if constant exposure is not maintained, toxicity may be underestimated. Reviews of published toxicity data for VCHs have identified that generally less than half of published data were usable as losses had not been prevented or actual exposure concentrations had not been measured (De Rooij et al. 1998; Zok et al. 1998). Closed, flow-through systems can be prohibitively expensive and are logistically difficult for testing micro-organisms and so, the current study used closed-static and semi-static renewal systems based on methods developed by Mayer *et al*. (2000) for algae.

The objectives of the current study were to determine: the suitability of jars (1 L) and vials (44 mL) in preventing the loss of VCHs during toxicity testing; and the suitability for toxicity testing with six indigenous Australian marine species.

MATERIALS AND METHODS

The suitability of the sealed test vessels for toxicity testing using six indigenous marine species was evaluated by assessing the survival in negative controls of artificial sea water (ASW) and filtered (at 0.45 µm) seawater (FSW). Tests for small organisms, namely micro-alga, and larvae of the sea urchin and oyster were undertaken in 44 mL clear glass vials with Teflon™ lined lids and no headspace. Toxicity tests with medium sized organisms, namely fish, amphipods and polychaetes were undertaken in 1 L glass jars sealed with Teflon™-lined lids containing approximately 500 mL water and 500 mL of headspace. Headspace was left in the jars to provide sufficient oxygen for the organisms. Toxicity test conditions for each of the test organisms are summarised in Table 1. For each test, temperature, pH, salinity and dissolved oxygen content of a sample from each treatment were measured at the start; immediately prior to renewal of test water; and at the conclusion of the test. Reference toxicants were undertaken for all tests with the exception of the juvenile polychaete worms (*D. dentata*), which had not previously been used as a test organism, and larval fish (*M. novemaculeata* – Australian Bass), to reduce the total number of organisms used in testing in accordance with the requirements of ethics approval granted for the project.

The first treatment was a complex mixture of VCHs obtained from contaminated groundwater at an industrial facility in Sydney, Australia. The groundwater sample was derived from two sources: shallow groundwater discharge collected from a stormwater drain and a groundwater sample from a nearby bundled piezometer. These samples were combined in a ratio of 9:1 (drain: piezometer), resulting in a concentration of approximately 100 mg/L of total VCHs. This manipulation was undertaken to ensure sufficiently high VCH concentrations to cause a response for all six test organisms. VCHs identified in the groundwater included: chloroform, vinyl chloride and tetrachloroethene; with 1,2-dichloroethane accounting for approximately 90% of the contaminant load by weight (Hunt *et al.* 2007). Seven serial dilutions of the groundwater sample were

Table 1. Summary of toxicity test conditions for six test organisms.

Test species	**Sea urchin** *Heliocidaris tuberculata*	**Rock oyster** *Saccostrea commercialis*	**Alga** *Nitzschia closterium*	**Australian Bass** *Macquaria novemaculeata*	**Polychaete** *Diopatra dentata*	**Amphipod** *Allorchestes compressa*
Test type	Static Non-renewal	Static Non-renewal	Static Non-renewal	Semi-static Renewal at 48 hours	Semi-static Renewal at 48 hours	Semi-static Renewal at 48 hours
Test Type[1]	Sub-chronic	Sub-chronic	Chronic	Acute	Acute	Acute
Test duration	72 hours	72 hours	72 hours	96 hours	96 hours	96 hours
Test end-point	Normal pluteus larvae	Larval development to D-veliger stage	Cell yield at 72-h	Imbalance, including survival	Survival	Survival
Test temperature	20 ± 1°C	20 ± 1°C	20 ± 1°C	20 ± 1°C	20 ± 1°C	20 ± 1°C
Test salinity	35 ± 1‰	35 ± 1‰	35 ± 1 ‰	35 ± 1 ‰	35 ± 1 ‰	35 ± 1 ‰
Test chamber	44 mL vial	44 mL vial	44 mL vial	1 L jar	1 L jar	1 L jar
Dissolved Oxygen Content (mg/L)	100.9 – 115.9	100.9 – 115.9	100.9 - 107.4	102.9 - 119.6	96.9 – 104.3	96.9-104.3
pH	7.6 - 8.3	7.6 - 8.3	7.6 - 8.3	7.5 - 8.1	7.7 - 8.1	7.7 - 8.1
Reference Toxicant Limits	7.5-10.1 µg Cu^{2+}/L	15.1-26.8 µg Cu^{2+}/L	19 - 24 µg Cu^{2+}/L[1]	Not Applicable	Not Applicable	0.84-5.4 mg NaDS[1]/L
Source of test organisms	Field collected, Sydney	Hatchery reared	CSIRO Marine Algal Supply Service	Hatchery reared	Hatchery reared	Field collected, Portarlington

[1] Range Identified in Hogan *et al.* (2005)

[2] NaDS (sodium dodecyl sulfate)

made, using filtered seawater, in twofold dilutions, with the highest and lowest proportions of groundwater being 50% and 0.75%, respectively. The salinity of the groundwater was adjusted to marine conditions (approximately 30 ppt) using artificial sea salts. Negative controls were included for artificial seawater (ASW; to a maximum proportion of 50%) and filtered seawater (FSW). The second and third treatments consisted of clean filtered seawater, individually spiked with 1,2-dichloroethane and chloroform. These two contaminants were selected as their concentrations in groundwater exceeded the ANZECC and ARMCANZ (2000) 95% TVs in estuarine waters (Hunt *et al.* 2007). Clean seawater was collected from Lurline Bay, a coastal site near Sydney and filtered to 0.45 µm. Chloroform and 1,2-dichloroethane were purchased from Lab Scan Analytical Services (AR Grade, 99.8% purity). For each chemical, a stock solution was prepared in seawater, the nominal concentrations of which were 1000 mg/L and 300 mg/L for 1,2-dichloroethane and chloroform, respectively. Each stock solution was serially diluted with seawater six times, by a factor of 3.

Samples were collected from test vessels to determine the potential loss of VCHs and inaccuracies during preparation of test solutions and during toxicity testing. To evaluate the loss of VCHs during preparation of test solutions, predicted (nominal) concentrations were compared to measured concentrations at the start of toxicity testing. Loss of VCHs during toxicity testing was evaluated by comparing measured concentrations of samples that were collected at the start (t = 0 h) and immediately prior to the renewal point (t = 48 h) of tests in jars, and at the start (t = 0 h) and end (t = 72 h) of tests in vials. To measure concentrations at the end of testing, an additional replicate vessel was prepared for each dilution. The vessel was filled with test solution and included in the incubator without test organisms. These samples represent the exposure concentration of organisms in the test vessels at the conclusion of testing (Table 2). Samples were collected and analysed from four of the seven dilutions in vials and all four treatments in jars (Table 2). Samples were collected in 40 mL glass vials with airtight Teflon™-lined lids with zero headspace, immediately preserved with hydrochloric acid and stored at <4°C. Samples were extracted using purge and trap methodology (USEPA 5030B) and analysed by Gas Chromatography and Mass Spectrometry (GC/MS) utilising a modification of USEPA Method 8260B for volatile organic compounds (USEPA 1996c). The two modifications

were a reduction in the number of analytes and surrogates, given the known contaminants in the groundwater and analytes were quantified by a single point calibration after validation against a compliant five point calibration. The modified method has been approved the National Association of Testing Authorities (Australia). The limit of reporting was 1 µg/L for all analytes with the exception of vinyl chloride (10 µg/L). Quality control evaluations indicated that no analytes were detected in method blanks and recoveries for laboratory control samples and matrix spikes and differences between primary and duplicate samples were within accepted criteria.

Relationships between initial nominal and measured exposure concentrations were derived using simple linear and polynomial regression analyses. Geometric means of the measured concentrations at the start and end of each toxicity test for each treatment were used as the measured exposure concentrations. Relationships between nominal and measured exposure concentrations were used to interpolate exposure concentrations where samples were not collected (i.e. for three of the seven dilutions in vials). Differences between measured concentrations at the start and end of toxicity tests (Table 2) were expressed as the relative percentile difference (RPD) (Equation 1).

$$\textbf{RPD = (Difference / Average) x 100} \qquad \textbf{(1)}$$

RESULTS AND DISCUSSION

Linear relationships between initial nominal and measured final VCH concentrations were derived for groundwater and polynomial relationships were derived for 1,2-dichloroethane and chloroform test solutions. All had coefficients of determination (r^2) of > 0.99; thus explaining more than 99% of the variation in measured concentrations. Loss of VCHs and inaccuracy during preparation of test solutions was evaluated by comparison of predicted (or nominal) concentrations and measured concentrations at the start of the toxicity testing. There was less than 50% difference between predicted and measured dilutions of contaminated groundwater in vials; however, for 1,2-dichloroethane and chloroform, differences between predicted and measured dilutions were between one and two orders of magnitude. In jars, difference between predicted and measured dilutions for contaminated groundwater, 1,2-dichloroethane and chloroform were all less than 50%.

Effects of the test solution preparation were larger for vials than jars, where considerably greater loss of VCHs was observed. The greater loss of VCHs for vials than for jars, could be explained by the greater number of dilutions required, i.e. seven in vials compared to four in jars. Smaller solution volumes were also required for vials than jars. The observed differences between predicted and measured concentrations highlight the need to undertake analytical testing to confirm exposure concentrations of VCHs, rather than relying on nominal exposure concentrations.

Measured concentrations for each of the three test solutions in vials indicated that there was no loss of VCHs for the duration of toxicity testing, with slight increases reported, within the range of analytical variability (Table 2). Average analytical variability for blind duplicates for this analysis at this laboratory is typically 25%, when measured by RPDs. The results likely reflect analytical variability rather than reflecting a true, and somewhat improbable, increase in analyte concentrations. In jars, measured concentrations at the conclusion and start of testing indicated a loss of VCHs in all but one sample treatment (300 mg/L dilution of 1,2-dichloroethane). Concentrations were lower at the end of testing by, on average, 29%, 52% and 57% for groundwater, 1,2-dichloroethane and chloroform,

Table 2. Nominal and measured concentrations (in mg/L) of test chemicals and volatile chlorinated hydrocarbons (VCHs) in vials and jars.

Treatment	Nominal Concentration	Measured Concentration		RPD
		Start	Final	
Vial	45	41.2	52.8	+24.8
Groundwater	10	9.22	10.2	+9.60
	2.5	2.12	2.37	+11.4
	0.5	0.45	0.68	+40.5
Vial	1000	811	1,140	+33.7
1,2-dichloroethane	100	88.1	130	+38.4
	10	5.40	5.83	+7.70
	1	0.057	0.063	+10.0
Vial	300	177	209	+16.6
Chloroform	30	9.58	10.9	+12.9
	3	0.229	0.335	+37.6
	0.3	0.003	0.005	+50.0
Jar	45	51.3	44.1	-15.1
Groundwater	19.5	19.8	16.5	-18.2
	8.5	10.1	7.25	-32.9
	3.5	4.67	2.74	-52.1
Jar	1000	516	368	-33.5
1,2-dichloroethane	300	150	158	+5.20
	100	69.6	51.2	-30.5
	30	18.5	13.2	-139
Jar	300	193	133	-36.8
Chloroform	100	49.1	27.1	-57.7
	30	29.4	11.9	-84.7
	10	10.7	6.42	-50.0

Table 3. Test acceptable criteria and survival of test organisms in artificial sea water (ASW) and filtered sea water (FSW) controls.

Organism	Test Acceptance Criteria	ASW control	FSW control	Reference Toxicant Result
Alga	Cell yield ≥ 30,000 cells/mL	64 250 cells/mL	58 250 cells/mL	22.7 µg Cu^{2+}/L
Fish	≥80% survival in controls	80% survival	**53% survival**	Not Applicable
Polychaete	≥90% survival in controls	100% survival	100% survival	Not Applicable
Sea Urchin	≥70% normal larvae in controls	91% normal	93% normal	9.1 µg Cu^{2+}/L
Oyster	≥70% normal larvae in controls	**69% normal**	83% normal	19.8 µg Cu^{2+}/L
Amphipod	≥90% survival in controls	**86% survival**	100% survival	3.53 mg NaDS/L

respectively. The RPD metric is skewed when low concentrations are present. Although these results include a component of analytical variability (~25%), they also likely represent an actual decline in VCHs and exposure concentrations of test organisms from volatilisation. Jars were less effective than vials at maintaining constant exposure concentrations for test organisms and preventing loss of VCHs. The greater losses of VCHs from jars than vials were probably due to compounds escaping into headspace present in the jars, in accordance with the findings of Mayer *et al.* (2000). Conditions with zero headspace, as was the case with vials in the current study, would, as long as the seal was airtight, prevent partitioning of contaminants into the vapour phase and subsequent loss from the test solution. Jars used in the current study for toxicity testing contained approximately 50% headspace allowing partitioning of VCHs into the vapour phase and loss from test solutions. Concentrations of chloroform declined more than VCHs or 1,2-dichloroethane. This is consistent with the finding of Mayer *et al.* (2000), that the lower the boiling point, the greater the loss from solution, as the boiling point of chloroform is approximately 30% lower than for 1,2-dichloroethane.

The suitability of the two types of test vessels for use with the test organisms was evaluated by survival of organisms in the two negative controls (i.e. artificial salt water – ASW and filtered salt water – FSW), in reference toxicants and maintenance of water quality parameters. In tests undertaken in both vials and jars, performance of reference toxicants were within the quality criteria (Table 3), dissolved oxygen content exceeded the minimum of 65% and pH was maintained within the required range (7.5 to 8.3) throughout all tests (Table 1). Micro-algal population growth and sea urchin larval development tests, both undertaken in vials, met the test acceptance criteria (Table 3). However, percent normal development in the oyster ASW control was 69%, marginally below the test criterion of 70% (Table 3) in the ASW control, which may indicate that the artificial salt used in the test is only marginally suitable for the oyster. The oyster toxicity test was also extended from 48 hours to 72 hours due to slow development of D-veliger shells, which often occurs in tests undertaken with this organism in winter, i.e. outside of the regular spawning season (Widdows 1993), as was the case in the current investigation. Overall, the 44 mL vials, sealed with Teflon™-lined lids and zero headspace, were suitable test

vessels for small organism tests. In jars, survival rates exceeded control limits with the exception of the fish larval imbalance test in the FSW control and the amphipod survival test in the ASW control (Table 3). Amphipod survival was 86%, marginally below the control limit of 90%, as was the case for the oyster, the artificial salt used in the test may only be marginally suitable for the amphipod. As survival in the FSW control and two treatments for both chloroform and 1,2-dichloroethane was 100%, this lower survival could be an anomalous result and unrelated to the use of jars. Fish survival was below the control limit of 80% in the FSW control (53%) (Table 3). Each replicate contains only five organisms and is therefore, sensitive to loss of one organism resulting in a lower survival rate (80%). Fish survival in the lowest concentration treatments of 1,2-dichloroethane and chloroform were 80% and 87% respectively, and therefore, met the control limits. Given this it is argued that the results of the fish toxicity test are valid. Previous fish larval imbalance toxicity tests with *M. novemaculeata* used larvae greater than 60 days old (Cohen and Nugegoda 2000). In the present test, however, in order to meet the recommended maximum of 30 days duration for an early life stage test (USEPA 2002), the larvae were 27 days old. This difference in age may have influenced survival in the controls. This is a sensitive test on an early life stage which requires further development.

All toxicity tests will periodically fail to meet their acceptability criteria. Therefore, by conducting one set of trials, we cannot categorically state that the test vessel is not appropriate for a particular species, particularly as they only just fail to meet the acceptability criteria. The fact that control values are so close to meeting the acceptability criteria indicates that (i) repetition would show the acceptability criteria are met in most cases or (ii) further modification may lead to the acceptance of the test vessel. Although previous studies have assessed the suitability of toxicity testing using algae in sealed vessels, the present study has shown that the use of sealed vessels for toxicity testing with VCHs is also suitable, based on the maintenance of suitable exposure conditions, for a diverse range of taxa including: urchins; bivalves; amphipods; and polychaetes, and potentially for fish larvae, for which further development of the test is required.

PAPER 3

DIRECT TOXICITY ASSESSMENT OF VOLATILE CHLORINATED HYDROCARBON CONTAMINATED GROUNDWATER AND DERIVATION OF A SITE-SPECIFIC GUIDELINE

ABSTRACT

Groundwater contaminated with a mixture of 14 volatile chlorinated hydrocarbons (VCHs) discharges to an estuarine embayment in Sydney, Australia. A screening-level hazard assessment identified a potential risk to aquatic organisms from surface water contaminated by the groundwater. Direct toxicity assessment (DTA) of the groundwater was undertaken on five indigenous marine species to assess toxicity and derive a site-specific guideline. The testing included acute tests, sub-chronic tests on early life stages and a chronic test. Test organisms included a micro-alga (*Nitzschia closterium*), an amphipod (*Allorchestes compressa*), a polychaete worm (*Diopatra dentata*), and sea urchin (*Heliocidaris tuberculata*) and oyster larvae (*Saccostrea commercialis*). Toxicity testing was undertaken in sealed containers to prevent loss of VCHs and concentrations of VCHs were measured to accurately assess exposure concentrations.

No observed effect concentration (NOEC) values varied from 1.56% dilution (1.11 mg total VCHs) to 50 % dilution (45.5 mg total VCHs). EC50 values varied from 4.8% dilution (3.77 mg total VCHs) to >50% dilution (45.5 mg total VCHs). NOEC data were used to derive species sensitivity distributions (SSD) and a site-specific guideline. SSDs were derived using Burr Type III (including the Pareto) and log-normal distributions. The log-normal distribution represented the best fit and as the Pareto distribution is a finite threshold model more suited to toxicants with a threshold mode of action, the log-normal SSD and the associated 95% trigger value (TV) of 830 µg/L of total VCHs, was adopted as the site-specific TV for the groundwater.

INTRODUCTION

Historic groundwater contamination with a complex mixture of 14 volatile chlorinated hydrocarbons (VCHs) was identified and extensively characterised at an industrial site in Sydney, Australia (1996) (see Figure 1). The contaminated groundwater was identified as migrating toward Botany Bay in southern Sydney. Its migration path intersected a stormwater system, causing contaminated groundwater to discharge to surface water in Penrhyn Estuary, an embayment in the northern margin of Botany Bay. A screening-level ecological hazard assessment by Hunt *et al.* (2007) identified surface water contamination in Penrhyn Estuary as posing a potential ecological hazard to aquatic organisms as concentrations of VCHs exceeded Australian and New Zealand Water Quality Guidelines (ANZECC and ARMCANZ 2000). The ANZECC and ARMCANZ (2000) Water Quality Guidelines (WQG) indicate that where trigger values (TVs) are exceeded, consideration should be given to site-specific factors including: background concentrations; locally important species; chemical and water quality modifiers; and mixture interactions (ANZECC and ARMCANZ 2000). The only modifier relevant to the current study is the presence of contaminant mixtures. The screening level hazard assessment (Hunt *et al.*, 2007) identified a greater hazard posed by the mixture of contaminants than by individual contaminants alone. As at least 14, potentially interacting chemicals are present in the mixture, the next step in the assessment framework is to undertake direct toxicity assessment (DTA) of the contaminated waters.

DTA is useful for monitoring effluents or complex mixtures in receiving waters (ANZECC and ARMCANZ 2000; Tinsley *et al.* 2004; Wharfe *et al.* 2004) and is akin to whole effluent toxicity (WET) testing undertaken for the assessment of toxicity of industrial effluent discharges in the United States (Grothe *et al.* 1996; USEPA 2000a) and the United Kingdom (Johnson *et al.* 2004; Tinsley *et al.* 2004). DTA is poorly developed in Australia compared to WET testing in Europe and the United States (ANZECC and ARMCANZ 2000). Whilst protocols in the United States are standardised, protocols have only been developed on a site-specific or regional basis in Australia (ANZECC and ARMCANZ 2000).

Some key advantages of DTA applicable to the present study are that it accounts for potential interaction between toxicants in a mixture of chemicals and the presence of toxicants that have not previously been identified in tested samples, neither of which would be accounted for by chemical testing alone (Wharfe 2004) or traditional single compound toxicity testing. Some limitations of DTA are a lack of adequate assessment of bioconcentration of hydrophobic contaminants, eutrophication of waters and potential for endocrine disruption (Waller *et al.* 1996). These limitations are not considered to be applicable to VCHs as these chemicals are not hydrophobic and do not bioaccumulate (McCarty and Mackay 1993b; Carey *et al.* 1998), do not interact with nutrients to cause eutrophication and have not been identified as potential endocrine disruptors (McCarty and Mackay 1993b; Carey *et al.* 1998). Most DTA and WET guidance recommends that a battery of test organisms be used to account for contaminants potentially having multiple modes of action (e.g. Johnson *et al.* 2004).Studies in the US have shown that prediction of adverse ecological effects is more accurate when a battery of test organisms is used (Diamond and Daley 2000).

SSDs are increasingly being used in Europe, the United States and more recently in Australia to derive risk-based environmental quality criteria to replace or complement the use of arbitrary assessment or safety factors (Posthuma *et al.* 2002). The SSD approach uses a probability distribution of effects to various organisms as a risk-based approach to derive numerical guidelines. The approach is an improvement over the use of arbitrary safety factors as it allows managers to choose a desired and risk-based level of protection. The limitations of safety factors are well documented (Chapman *et al.* 1998; Warne 1998).

SSDs are typically derived for national WQGs and regional frameworks, however, assessments using site-specific SSDs are rare. An assessment undertaken by Bossuyt *et al.* (2005) found no difference between site-specific and regional SSDs for copper and zinc, which is consistent with the conceptual underpinning of SSDs. In Australia, derivation of site-specific guidelines is recommended where existing data are

insufficient or inappropriate (NEPC 1999; ANZECC and ARMCANZ 2000). At the time of writing the guidelines, the derivation of site-specific guidelines from DTA was commonly undertaken by application of safety factors to NOEC data, however, the guidelines allowed for a flexible approach, dependent on available data (Chapman 2001). Since then a number of site-specific guidelines have been derived and given regulatory endorsement, but essentially none have been published.

The VCHs present in groundwater in the current study, predominantly chloroethenes and chloroethanes, have a narcotic mode of action (Di Toro and McGrath 2000; Di Toro *et al.* 2000; Escher and Hermens 2002). Narcosis, or baseline toxicity, is the result of partitioning of pollutants into biological membranes followed by non-specific disturbance of membrane integrity and function (van Wezel and Opperhuizen 1995; Carey *et al.* 1998). The effects of narcosis are reversible (Escher and Hermens 2002) and have been observed in all types of organisms, including plants, bacteria, vertebrates and invertebrates (Carey *et al.* 1998). For Type I narcosis, toxicity is a function of the tendency of the contaminants to dissolve into chemical membranes, which in turn, is a function of the octanol water partitioning coefficient of the chemical (K_{ow}). As VCHs are water soluble and do not bioaccumulate, it is appropriate to derive a site-specific guideline based on the results of toxicity testing.

The objectives of the current study were: to undertake DTA of contaminated groundwater containing VCHs using five indigenous marine species to assess potential toxicity and derive a site-specific guideline using the SSD approach; and to asses the influence of the selection of input parameters on the resulting SSDs and TVs.

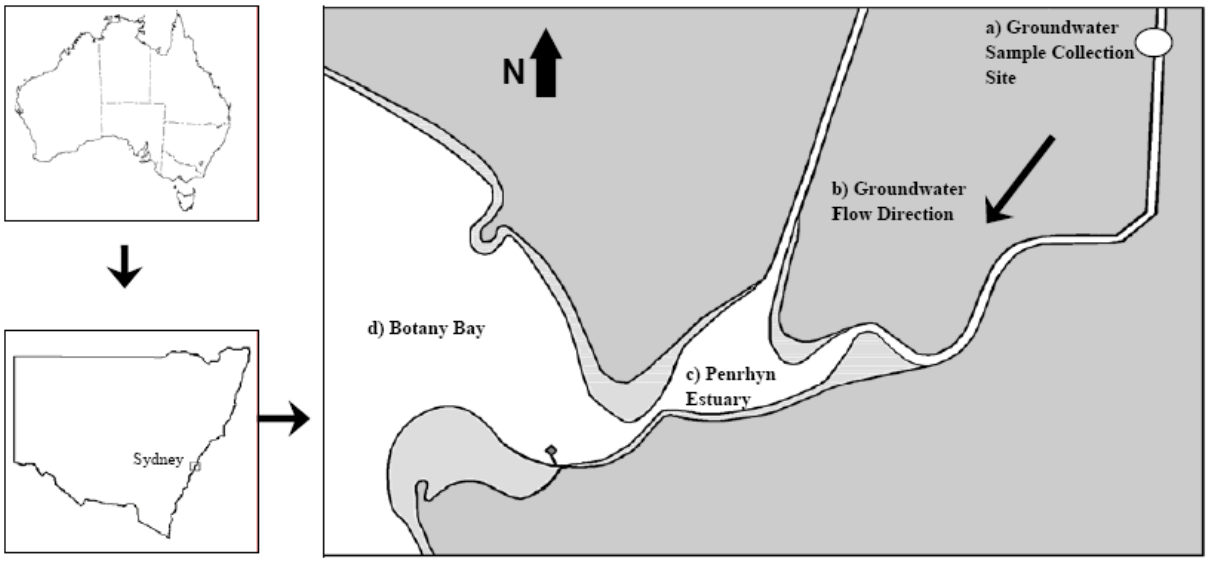


Figure 1. Location plan of Penrhyn Estuary, Sydney, Australia indicating a) the groundwater sample collection site, b) groundwater flow direction and receiving waters in c) Penrhyn Estuary and d) Botany Bay.

METHODOLOGY

TEST WATER PREPARATION

Contaminated groundwater was collected from two sources: shallow groundwater discharge from a stormwater drain; and a sample from a nearby piezometer, both upgradient of the receiving ecosystem, Penrhyn Estuary, Sydney, Australia (Fig. 1). These two samples were combined in a ratio of 9:1 (drain:piezometer) resulting in a concentration of approximately 100 mg/L of total VCHs (see Results). This manipulation (i.e. addition of groundwater from the piezometer) was undertaken to ensure sufficiently high VCHs were present to elicit a response in all test organisms and was done immediately prior to preparation of the groundwater dilutions for toxicity testing. The salinity of the groundwater mix was adjusted to 30 ppt using artificial sea salts in order to ensure satisfactory test conditions for test organisms and to represent the marine conditions of the receiving ecosystem. Dilution seawater was collected from a clean site at Lurline Bay, Sydney, Australia and filtered to 0.45µm.

THE NUMBER AND SELECTION OF TEST SPECIES

The toxicity of the contaminated groundwater was assessed using five indigenous marine species that belong to 5 taxonomic groups of organisms. This meets the minimum data requirements to use a SSD (i.e. at least 5 species belonging to at least 4 different taxonomic groups) set by (ANZECC and ARMCANZ 2000).

The battery of test organisms selected in the current study represents organisms that are present in the receiving environment during at least some part of their life stages, are ecologically relevant and some have commercial or recreational value in the area. *Saccostrea commercialis* (Sydney Rock Oyster) is farmed and collected on the southern shores of nearby Botany Bay. Amphipods, including *Allorchestes compressa*, are the dominant macroscopic group on reef surfaces and are consumed in great quantities by larger organisms. This animal is also the dominant component of the diets of small (0.1 to 100 g) inshore fishes (Edgar 1997). *Heliocidaris tuberculata* (sea urchin) and *Diopatra dentata* (polychaete worm) are both commonly found in the Botany Bay. The

test animals are also from a variety of trophic levels i.e. primary producers (*N. closterium*), grazers (*H. tuberculata* and *A. compressa*), a filter feeder (*S. commercialis*) and a detritivore (*D. dentata*). As narcosis is the mode of action for VCHs, all test species should be sensitive to the contaminants.

TOXICITY TESTING

VCHs would be lost quickly from the groundwater samples if test vessels were left open to the atmosphere. Toxicity tests were therefore, undertaken in sealed vessels to prevent loss of VCHs and to maintain constant exposure concentrations. Previous studies that have used closed flasks to prevent loss of volatile contaminants have focussed on micro-algae (Galassi and Vighi 1981; Herman *et al.* 1990; Mayer *et al.* 2000) or cladocerans (Rose *et al.* 1997). In the current study, closed containers were used for algae, amphipods, juvenile polychaetes and urchin and oyster larvae, the methodology for which was evaluated in Hunt *et al.* (2009a). General characteristics of the methods are provided below followed by details of the methods for each species.

Toxicity testing of small organisms (i.e. urchin and oyster larvae and the alga) was undertaken in 44 mL glass vials with Teflon™ lined lids and zero headspace. Seven dilutions, each conducted in quadruplicate, were tested, i.e. 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5% and 0.75% of the 9:1 groundwater mixture. These solutions were not renewed during the tests (72 h duration). Toxicity tests with larger organisms (i.e. amphipods and juvenile polychaetes) were undertaken in 1 L jars with 500 mL of groundwater and sealed with Teflon™ lined lids. Four dilutions, each conducted in triplicate, were tested, i.e. 50%, 25%, 12.5% and 6.25% of the 9:1 groundwater mixture. Test solutions in jars were renewed at the mid point of testing (i.e. 48 h). Toxicity test conditions are summarised in Table 1. Filtered seawater (FSW) and artificial seawater (ASW) controls were undertaken for each toxicity test. Temperature, pH, salinity and dissolved oxygen content of a representative sample from each treatment were measured daily.

Table 1 Summary of toxicity test conditions.

Test species	**Sea urchin** *Heliocidaris tuberculata*	**Rock oyster** *Saccostrea commercialis*	**Benthic Alga** *Nitzschia closterium* (CSIRO Strain CS-5)	**Polychaete** *Diopatra dentata*	**Amphipod** *Allorchestes compressa*
Test type	Static, non-renewal	Static, non-renewal	Static, non-renewal	Static, renewal at 48 hours	Static, renewal at 48 hours
Test duration	72-hour	72 hours	72-hour	96-hour	96-hour
Test end-point	Normal pluteus larvae	Larval development to D-veliger stage	Cell yield at 72-h	Survival	Survival
Test temperature	20±1°C	20±1°C	21 ± 1°C	20 ± 1°C	20 ± 1°C
Test salinity	35±1‰	35±1‰	35 ± 1 ‰	35 ± 1 ‰	35 ± 1 ‰
Test chamber size / volume	44 mL glass vial with zero headspace	44 mL glass vials with zero headspace	44 mL glass vials with zero headspace	500 mL in 1 L glass jars with Teflon™ lined lids.	500 mL in 1 L glass jars with lids.
Source of test organisms	Field collection, Sydney coastal region	Oyster farms / hatchery reared	CSIRO Marine Algal Supply Service (Strain CS-5) in Hobart, Tas.	Aquabait Pty Ltd, Dora Creek, NSW	Field collected, Portarlington, Victoria
Test concentrations Effluent (%)	0.75%, 1.5%, 3.1%, 6.25%, 2.5%, 25% and 50%.			6.25%, 12.5%, 25% and 50%	

The 72-h sea urchin larval development test was undertaken using *H. tuberculata*. The test endpoint was the percent normal development of pluteus larvae. The procedure used was based on methods described in USEPA (1994) and ASTM (1995) and adapted for use with *H. tuberculata* by Doyle *et al.* (2003). Adult sea urchins were collected from Lurline Bay, Sydney, NSW, transported to the laboratory and spawned within 6 hours. Only adult organisms were used to ensure reproductive maturity. Spawning was induced by injecting 2 mL of 1 M KCl solution into the peristomal cavity. Once spawning commenced and the sex of organisms was determined, organisms were separated. Females were inverted in a glass bowl of seawater to allow discharge of eggs, which were collected and stored in filtered fresh salt water (FSW). Sperm from male urchins was collected dry using a pipette to prevent activation and stored at 4^{o}C in a glass vial until required for fertilisation (<1 hour). Viable gametes were selected on the basis of fertilisation success trials and visual examination of gamete maturity. Eggs were fertilised at an egg:sperm ratio of approximately 1:100, and eggs were introduced into the test vials at a rate of 35 eggs/mL. After the 72 h exposure period, buffered formalin was added to each test vessel. One mL of test solution was drawn directly from the bottom of each test vessel and placed in a Sedgwick-Rafter counting chamber. The first 100 larvae were examined and the numbers of normal and abnormal larvae, based on His *et al.* (1999), were recorded.

The 72-h oyster larval development toxicity test was undertaken using larvae of the rock oyster *S. commercialis* based on methods described by USEPA (1996a) and APHA (1998) and adapted for use with *S. commercialis* by Krassoi (1996). The test endpoint was the percent normal development of D-veliger stage larvae and is normally conducted over a 48 h period. However, as the testing was conducted outside the normal spawning season, the test exposure period was extended to 72 h to allow at least 70% of embryos to reach the normal D-veliger stage (Widdows 1993). Oysters were obtained from a clean site at Wallis Lake, NSW. Oysters were spawned by gonad stripping, and viable gametes selected on the basis of fertilisation success trials and visual examination of gamete maturity. Eggs were fertilised by adding spermatozoa to the egg suspension so that the final egg: sperm ratio was 1:100. Density of the egg suspension was determined using a Sedgwick-Rafter counting chamber to determine the volume

required to achieve a final density of 100 eggs/mL. Test vials were inoculated with 500 ± 50 eggs within 2 h of fertilisation. After 72 h exposure, buffered formalin was added to each vessel. One mL of test solution was drawn directly from the bottom of each test vessel and placed in a Sedgwick-Rafter counting chamber. The first 100 oyster larvae were examined and the number of normal and abnormal D-veliger larvae was recorded in accordance with Krassoi (1996).

The 96-h polychaete toxicity test used juveniles of the polychaete *D. dentata* and was undertaken based on methods described by APHA (1998) and USEPA (1994, 1996b). The test endpoint was the percent survival of juvenile organisms at 96 hours. Juvenile polychaetes, 3 to 5 months old were purchased from Aquabait Pty Ltd, Dora Creek, NSW. *D. dentata* is abundant along the NSW coastline in shallow sandy environments (Edgar 1997). *D. dentata* has not been used as a test organism previously. Five individuals were randomly selected and introduced into each 1 L jar. Jars were examined every 24 h and numbers of surviving polychaetes recorded.

The 72-h micro algal growth inhibition (cell yield) test using *N. closterium* was based on methods described by USEPA (1996b) and Stauber *et al.* (1994). The test endpoint was cell yield at 72 hours. *N. closterium* is a unicellular estuarine diatom which was initially isolated from Port Hacking and reared in the CSIRO Marine Algal Supply Service (Strain CS-5) in Hobart. Organisms were supplied in log growth phase and used in accordance with the standard protocol for the test (Stauber *et al.* 1994). Guillards™ F/2 nutrient stock solutions were added to each test and control treatments to provide nutrients required for micro algal growth. Micro algae used to inoculate the test vessels were concentrated from cultures in log-growth phase by centrifugation, and re-suspended using dilution water. This process was repeated a second time to remove the original culture medium. The density of micro algae was determined using an Improved Neubauer Haemocytometer and test vessels were inoculated with micro algae such that the final concentration at t = 0 was approximately 10,000 cells/ml. Test vials were incubated for 72-h in a constant temperature cabinet equipped with cool-white fluorescent tubes to provide 5000 ± 500 Lux continuous lighting. At the end of the

incubation period, three counts of algal density were made using an Improved Neubauer Haemocytometer for each replicate and recorded as the number of cells per µL.

The 96-h amphipod acute toxicity test using juveniles of *A. compressa* was undertaken based on methods described by APHA (1998) and USEPA (1994, 1996b). The test endpoint was the percent survival of juvenile organisms at 96 h. *A. compressa* has previously been used in the assessment of effluent toxicity in the Sydney area (AWT ES&T 1996; Woodworth *et al.* 1999). Juvenile amphipods (approximately 2-5 mm in length) were collected from Portarlington, Victoria and held in aquaria in the laboratory until required for testing. Five individuals were randomly selected and introduced into each 1 L jar. Jars were examined every 24 h and numbers of surviving amphipods recorded.

MEASUREMENT OF EXPOSURE CONCENTRATIONS

Concentrations of VCHs were measured by collection and analysis of samples from test vessels at the start and end of testing in accordance with the methodology presented in Hunt *et al.* (2009a). To allow assessment of potential toxic effects in the receiving ecosystem, percentage groundwater was correlated with the concentration of total VCHs. Samples were collected in 40 mL glass vials with airtight Teflon™ lined lids with zero headspace. The samples were preserved immediately with hydrochloric acid and stored at less than 4°C. Samples were extracted using purge and trap methodology (USEPA 5030B) and analysed by Gas Chromatography Mass Spectrometry (GC/MS) utilising a modification of the USEPA Method 8260B for volatile organic compounds (USEPA 1996c). The limit of reporting was 1 µg/L for all analytes, with the exception of vinyl chloride (10 µg/L). Quality control evaluations were undertaken on each sample batch. No analytes were detected in the method blanks and recoveries for laboratory control samples and matrix spikes were between 80% to 120%, and within the accepted criteria. Differences between primary and duplicate samples were generally less than 25%, which was considered acceptable (Hunt *et al.* 2009a). Relationships between percent dilution and concentration of total VCHs for the vials and the jars was presented in Hunt *et al.* (2009a). The geometric mean between the start

and end concentrations was adopted to represent the exposure concentration in each dilution. Logarithmic transformations were undertaken before derivation of linear relationships between dilution of groundwater and concentration of VCHs. These relationships were used to transform the NOEC, LOEC and EC50 metrics from percent dilution to total VCHs.

Calculation of Toxicity Metrics

Concentrations of groundwater affecting 50% of test organisms (LC50 and EC50 values) were determined by the trimmed Spearman-Karber Method using TOXCALC™ v5.0 (Tidepool™ Scientific Software). No observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values were determined by performing Dunnett's or Steel's Many-One Rank tests, depending on the distribution of the data using TOXCALC™ V5.0 (Tidepool™ Scientific Software).

SSD and Site-Specific Guideline Derivation

The SSD method used to derive WQGs in Australia, New Zealand and South Africa fits a Burr Type III distribution that best fits the available toxicity data (Shao 1990). This is done by the BurrliOZ™ software (Campbell *et al.* 2000). The Burr Type III distribution is a flexible three-parameter (b, c and k) distribution that provides good approximations to the commonly used log-logistic, log-normal, log-triangular and Weibull distributions (Shao 1990). For the Burr Type III distribution, as $k \rightarrow \infty$ the distribution tends to the Reciprocal Weibull distribution and as $c \rightarrow \infty$ the distribution tends to the Reciprocal Pareto distribution. In some cases, where a suitably accurate Burr Type III distribution cannot be fitted, the BurrliOZ™ program will discard the Burr Type III distribution and fit a Reciprocal Weibull or Reciprocal Pareto distribution (Campbell *et al.* 2000). If visual assessment of the BurrliOZ™ plots indicates that a distribution other than the selected Burr Type III distribution fits the data better, then the ETX™ and BurrliOZ™ programs, or other appropriate software, should both be used. The fit of the log-normal (ETX™) and Burr Type III (BurrliOZ™) distributions should then be assessed by analysis of the correlation between observed and predicted toxicity for each model, and the best fitting distribution should be adopted. Given the dataset only contains 5 species, an *a priori* decision was made to calculate all PC values using both BurrliOZ™ and

ETX™ programs and adopt the PC values generated by the distribution that best fits the data.

Toxicity data are manipulated before being used in the derivation of SSDs. Two such manipulations are the classification of data as acute or chronic and the size of the ACR used to convert acute data to estimates of chronic toxicity. Whilst guidance provided in ANZECC and ARMCANZ (2000) indicates that it is preferable that chronic data rather than acute data be used in the derivation of guideline values, there is a shortage of available indigenous chronic tests (van Dam and Chapman 2001). It is also not entirely clear whether the sea urchin and oyster early life stage (ELS) tests are acute or chronic. For example, the Australian and New Zealand WQGs (ANZECC and ARMCANZ, 2000) consider tests with an exposure duration of ≤96 h to be acute, unless the test organism is a micro-organism, in which case, durations of ≥72 h are considered chronic. In contrast, others (e.g. (USEPA 2002; Stauber 2003; Warne 2008) consider ELS test data as chronic. There is similar uncertainty regarding the size of the ACRs to be used. The default ACR used by ANZECC and ARMCANZ (2000) is 10. However, di Toro *et al.* (2000) and McGrath *et al.* (2004) found ACRs for non-polar narcotic contaminants to be closer to 5, with estimations of 4.5 ± 2.5 and 5.09 ± 0.95, respectively.

In the current study, an ACR of 5 was adopted for acute EC50 data, in accordance with di Toro *et al.* (2000) and McGrath *et al.* (2004), and the two ELS tests (i.e. urchin and oyster larval development tests) were treated as chronic tests in the derivation of the site-specific SSD. However, to evaluate the sensitivity of the SSD and the resulting concentrations that should theoretically protect 95% of species (i.e. PC95 values) to including test results as acute or chronic and the choice of ACR (of either 5 or 10 for acute EC50 data), an additional three scenarios were modelled. The first additional scenario was the same as the original except that for the acute tests an ACR of 10 was applied. In the second additional scenario, the ELS tests were treated as acute tests and an ACR of 5 was applied to all the acute test data, while in third additional scenario the ELS tests were treated as acute tests and an ACR of 10 was applied.

RESULTS

CHEMISTRY

The composition of groundwater was dominated by 1,2-dichloroethane, which accounted for approximately 90% of the total composition by weight, which equates to approximately 45 mg/L of total VCHs in the 50% dilution of the groundwater mixture (Table 2). The groundwater contains a mixture of 14 VCHs (Hunt *et al.* 2007) including: 1,2-dichloroethane; chloroform; tetrachloroethene; carbon tetrachloride; and vinyl chloride. Strong linear relationships between the percent dilutions were identified in vials ($y = 1.0513x + 11.427$; $r^2 = 0.99$; $n = 4$) and jars ($y = 0.6066x + 11.146$; $r^2 = 0.99$; $n = 4$). Exposure concentrations measured in vials indicated that there was no measurable loss of VCHs over the testing period. However, losses of 30%, on average, were measured in jars (Hunt *et al.* 2009a).

TOXICITY

The responses of various species to the groundwater are shown in Table 3, while the toxicity estimates are shown in Table 4. In the algal growth test, growth was significantly lower in the 1.5% groundwater dilution than the controls ($P<0.05$)(Table 3). Of the four replicates, three reported cell densities of between 5.3×10^4 and 5.7×10^4, whilst one replicate reported growth of 2.0×10^4. As the population growth in the 3% groundwater treatment was not significantly different ($P<0.05$) from the controls (average of 5.9×10^4), the low growth in the 1.5% dilution may be a result of inadequate inoculation with either cells or the Guillards™ F/2 culture medium. The 3% groundwater dilution (2.30 mg/L total VCHs) was adopted as the NOEC (Table 4).

The rock oyster larval development toxicity test did not meet all quality assurance criteria. The mean percentage of normally developed D-veliger larvae in the ASW control was 68.6%, marginally less than the minimum control criteria of 70% (Table 3).

Table 2. Volatile chlorinated hydrocarbons (VCHs) in the 50% dilution of the groundwater mixture and available ANZECC and ARMCANZ (2000) trigger values.

Analyte	Trigger Value (µg/L)	50% Effluent (µg/L)
Carbon tetrachloride	240	416
Chloroform	370	594
1.1.2.2-tetrachloroethane	400	45
1.1.2-trichloroethane	1,900	146
1.1-dichloroethane	*1,450*	33
1.2-dichloroethane	1,900	44,100
Tetrachloroethene	70	674
Trichloroethene	330	416
1.1-dichloroethene	*3,900*	24
cis-1.2-dichloroethene	*1,250*	447
Vinyl chloride	100	675
Total VCHs	--	47,570

Trigger values in italics were presented in Hunt *et al.*, (2007)

-- Denotes that a Trigger Value for Total VCHs is not available

Table 3. Toxicity test results of direct toxicity assessment of contaminated groundwater.

	N.closterium Alga	*H.tuberculata* Sea Urchin	*S.commercialis* Oyster	*D.dentata* Polychaete	*A.compressa* Amphipod
Concentration %			**Mean Response (±S.E.)**		
FSW control	91%±14%	93%±1%	83%±2%	100%±0%	100%±0%
ASW control	100%±3%	91%±1%	69%±2%	100%±0%	86%±6%
Control Limit	Minimum Yield 30,000 cells/mL	70% normal development	70% normal development	90% survival	90% survival
Effluent Dilution					
0.78%	100%±7%	103%±1%	93%±5%	--	--
1.56%	**73%±14%**	100%±1%	97%±5%	--	--
3.13%	92%±1%	**59%±2%**	102%±4%	--	--
6.25%	**45%±9%**	**44%±10%**	98%±3%	100%±0%	108%±6%
12.50%	**13%±7%**	**12%±3%**	**44%±5%**	100%±0%	115%±0%
25.00%	0%±0%	0%±0%	0%±0%	67%±33%	115%±0%
50.00%	0%±0%	0%±0%	0%±0%	0%%±0%	92%±12%

-- *Indicates that dilutions were not tested*

Results shown in **bold** were statistically different from both controls

Table 4. Summary of NOEC, LOEC and EC50 toxicity metrics derived from direct toxicity assessment of groundwater mixture as percent dilution of the groundwater mixture and as concentrations of total volatile chlorinated hydrocarbons (VCHs).

Groundwater Dilution		Alga	Urchin	Oyster	Polychaete	Amphipod
Dilution (as %)	NOEC	3.13	1.56	6.25	25.00	50.00
	LOEC	6.25	3.13	12.50	50.00	>50.00
	EC50	5.20	4.80	11.90	28.10	>50.00
	EC50 95% LCL	2.95	4.55	11.22	23.71	--
	EC50 95% UCL	9.05	5.07	12.55	33.22	--
Concentration of total VCHs in mg/L	NOEC	2.30	1.11	4.98	29.88	45.50
	LOEC	4.98	2.30	10.31	45.50	45.50
	EC50	4.10	3.77	9.79	32.08	>45.50
	EC50 95% LCL	2.32	3.57	9.23	27.16	--
	EC50 95% UCL	7.13	3.98	10.32	38.05	--

95% LCL - lower 95% confidence limit

95% UCL – upper 95% confidence limit

-- No confidence limits applicable

For the amphipod (*A. compressa*) testing, the NOEC was 50% groundwater dilution (45.50 mg/L total VCHs). As this was the highest concentration tested, the LOEC was >50% (>45.50 mg/L total VCHs). No LC50 was estimated as there were no observed effects in the range tested. The mean percentage survival was 87% in the ASW control, marginally below the minimum control survival criteria of 90%. Given the 100% survival in the exposure treatments, this was considered acceptable

NOECs for the five species tested varied from 1.56% groundwater dilution (1.11 mg/L total VCHs) for the sea urchin larval development to 50% groundwater dilution (45.5 mg/L total VCHs) for the amphipod survival test (Table 3). The LOEC values ranged from 3.13% groundwater dilution (2.30 mg/L total VCHs) for the sea urchin to >50.00 % groundwater dilution (>45.50 mg/L total VCHs) for the amphipod. The EC50 values varied from 4.8% groundwater dilution (3.77 mg/L total VCHs) for the sea urchin larval development test to >50% groundwater dilution (>45.5 mg/L total VCHs) for the amphipod survival test (Table 4).

SSD AND SITE-SPECIFIC GUIDELINE DERIVATION

The BurrliOZ™ software used in the current study could not fit a suitable Burr Type III curve (as $c \rightarrow \infty$) and therefore, the curve was replaced with the best-fitting Reciprocal Pareto distribution. The PC95 values for the Reciprocal Pareto (Figure 2) and log-normal (Figure 2) distributions were 639 µg/L total VCHs (rounded to 640 µg/L) and 829 µg/L total VCHs (rounded to 830 µg/L), respectively (Table 5). In addition to the Reciprocal Pareto distribution, BurrliOZ™ also fitted log-normal and log-logistic distributions to the toxicity data (Figure 2). Correlations between each of the Reciprocal Pareto and log-normal distributions and the original test data were derived. Correlations for the Reciprocal Pareto distribution was $R^2 = 0.84$ and for the corresponding log-normal distributions, was $R^2 = 0.89$. The log-normal SSD passed the Anderson-Darling test for normality ($P<0.01$).

The statistical distributions fitted to the toxicity data for the three additional scenarios were the Reciprocal Pareto; Burr Type III; and log-normal distributions (Table 5). PC95

values derived using the BurrliOZ SSD method varied from 220 µg/L to 930 µg/L total VCHs while those derived by ETX™ varied from 275 µg/L to 965 µg/L total VCHs (Table 5). The site-specific SSD included treatment of larval development tests as chronic tests (i.e. no ACR applied) and applied an ACR of 5 to acute tests, however, when the ACR was changed from 5 to 10, PC95 values estimated by the log-normal and Pareto distributions increased by 15% and 50%, respectively (Additional Scenario 1 – Table 5). When the ACR was maintained at 5 and the larval development tests were treated as acute tests (i.e. ACR applied), PC95 value estimated by the log normal distribution decreased by 50% compared to the original scenario, whilst the PC95 value estimated by the Burr type III distribution increased compared to the original scenario by 5% (Additional Scenario 2 – Table 5). When the ACR was changed from 5 to 10 and the larval development tests were treated as acute and not chronic, PC95 values estimated by both log-normal and Reciprocal Pareto distributions decreased by approximately threefold (Additional Scenario 3 – Table 5) compared to the original scenario.

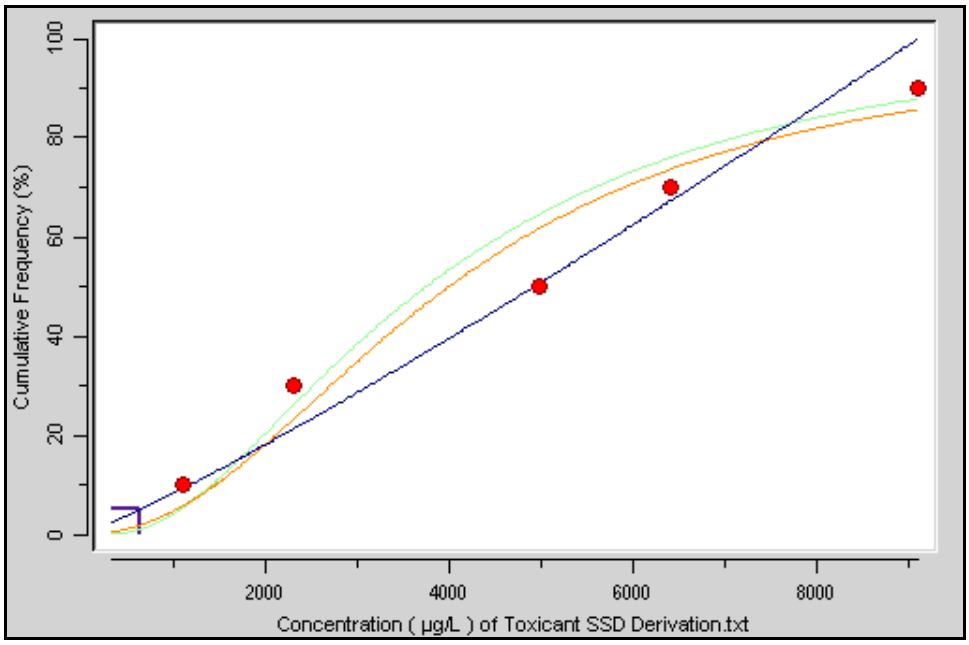


Figure 2. Species sensitivity distributions derived using BurrliOZ™ software for groundwater including the Reciprocal Pareto (r^2=0.84) (blue line), log-normal (r^2=0.88) (green) and log-logistic (orange) distributions. Red circles represent individual NOEC data points.

Table 5. Estimate of PC95 values (µg/L) for groundwater mixture containing volatile chlorinated hydrocarbons.

	BurrliOz™		ETX™				
	PC95	Distribution Type	PC95	Distribution Type	ACR	Treatment of Larval tests	Input Data
Original Scenario	640	Reciprocal Pareto	830	Log-normal	5	Chronic	2300, 1110, 4975, 6416, 9101
Additional Scenario 1	930	Reciprocal Pareto	965	Log-normal	10	Chronic	2300, 1110, 4975, 3208, 4550
Additional Scenario 2	680	Burr Type III	490	Log-normal	5	Acute	2300, 754, 1958, 6416, 9101
Additional Scenario 3	220	Reciprocal Pareto	275	Log-normal	10	Acute	2300, 377, 979, 3208, 4550

DISCUSSION

The survival in each of the ASW controls for the amphipod and oyster larval development tests were marginally (i.e. <5%) below the acceptance criteria. However, this does not affect the reliability of the toxicity data as the tests were conducted using filtered sea water as the dilution water. It does, however, indicate that the use of artificial sea salts as dilution water may not be suitable for all marine test organisms. The organisms in the study exhibited a wide range of sensitivity with NOECs ranging from approximately 1 mg/L to > 45 mg/L total VCHs). The urchin larval development test was consistently the most sensitive test with the amphipod the least sensitive test. The order of decreasing sensitivity of tests, for both NOEC and EC50 data was urchin larval development > algal population growth > oyster larval development > polychaete juvenile survival > amphipod survival. Toxicity metrics including NOEC and EC50 and derived PC95 values were derived as concentrations of total VCHs, as this is more readily measurable and environmentally relevant than percent dilution.

SSDs for the site-specific guideline were derived with PC95 values of 640 µg/L total VCHs (Reciprocal Pareto) and 830 µg/L total VCHs (log-normal)(Table 5). Correlation between the predicted toxicity and the observed NOEC data indicated that the log-normal distribution was a marginally better fit than Reciprocal Pareto distribution, accounting for 89% of the variability. The Reciprocal Pareto distribution, however, is a finite threshold model, which is more suitable to fitting threshold toxicants such as copper (Brix *et al.*, 2001) and zinc (van Sprang *et al.*, 2004). The log-normal model is a continuous distribution, which is more suitable for the toxicants in this study (VCHs), which do not have a threshold mode of action. Based on the above, it is recommended that the log-normal distribution, with the associated PC95 of 830 µg/L total VCHs, derived using an ACR of 5 and treating larval development tests as chronic tests, should be adopted as the site-specific guideline for the groundwater. The log-normal distribution is favoured by some workers because of the strong existing mathematical basis for its interpretation (Duboudin *et al.* 2004). Despite the various preferences of individuals or organisation there is no theoretical basis for assuming the SSD should conform to any particular distribution (Forbes and Forbes 1993). Newman *et al.* (2000a) evaluated a non-parametric

bootstrapping methodology, however, the results of this were similar to the log-normal model anyway. Newman *et al.* (2000a) concluded that although there are shortcomings associated with the assumption of distributions for SSDs, the SSD approach provided a pragmatic method of ERAs moving forward beyond the hazard quotient (HQ) method.

In the current study, the PC95 value derived using the log-normal distribution was 830 µg/L total VCHs. The current Australian trigger values (TVs) for slightly to moderately modified water bodies (i.e. PC95) and site specific PC95 values for VCHs vary from 100 µg/L for vinyl chloride to 3900 µg/L for 1,1-dichloroethene (ANZECC and ARMCANZ 2000; Hunt *et al.* 2007). When the TVs are reviewed using the toxic unit (TU) approach, (i.e. accounting for composition of the VCHs being ~90% 1,2-dichloroethane (on a mass basis) and ~10% for the remaining components), the resulting TV for total VCHs in the groundwater would be ~1800 µg/L. The derived PC95 values for the VCH mixture in the groundwater were always considerably lower than those derived using the TU approach. Assuming the various TVs are correct, this suggests that either there are other chemicals present which have not been accounted for or that the overall form of interaction between the chemicals is more than additive.

The standard deviation of the log-normal SSD derived in the present study and adopted for the site-specific guideline was 0.37, approximately half of the standard deviation of SSDs of 0.69 and 0.71 for narcotic contaminants derived by De Zwart (2002) and McGrath *et al.* (2004) for narcotic contaminants. The smaller standard deviation of the SSD indicates that the curve was considerably steeper, with less variability in species sensitivity and possibly not representative of a typical narcotic distribution. The difference in the standard deviations between the adopted SSD and standard SSDs for narcotic contaminants may be a product of the small dataset used in the study or an underlying difference in toxicity characteristics of the mixture. The small number of test species also increases the variability around the estimate of the hazardous concentration to 5% of organisms (HC5) (830 µg/L), with the lower and upper limits of the HC5 being 105 µg/L and 1875 µg/L, respectively.

The availability of suitable indigenous test organisms greatly affected test species selection, test methods and test endpoints. It has been suggested that organisms for toxicity testing, particularly in DTA studies should be selected from the receiving environment and not from a set of traditional test organisms in order to reduce potential bias towards a small set of easily reared and proven organisms and increase the validity and relevance of the testing program (Kefford *et al.* 2005). The Australian and New Zealand WQGs provide a flexible approach for the derivation of TVs, dependent on the data available and where sufficient data are available, the preferred method is the SSD approach (Chapman 2001). Work undertaken by Newman *et al.* (2000b) has shown that the optimum number of species is between 10 and 30. Undertaking toxicity testing on this number of species is, however, a major undertaking, is arguably not appropriate for a site-specific assessment and given the lack of available chronic indigenous test organisms available (van Dam and Chapman 2001) would not be possible. Of the five species used in the present study, four are routinely used test organisms (*N. closterium*, *A. compressa*, *S. commercialis* and *H. tuberculata*) and one has not previously been used as a test organism (*D. dentata)*, however, all of the test species are considered representative of the receiving ecosystem. The ANZECC and ARMCANZ (2000) WQGs indicate that to derive a site-specific guideline value, it is desirable to have greater than five chronic tests, however, the choice is greatly restricted by the small number of indigenous organisms with suitable chronic tests available (van Dam and Chapman 2001). The five species chosen were considered to be representative of the receiving ecosystem as all are temperate marine species that are likely to be present in the receiving waters for at least part of their life stages. The social and economic relevance of the test species, their sensitivity to the toxic mode of action and the testing of several different trophic levels, also make the battery of test organisms suitable for derivation of site-specific guidelines for this ecosystem. Development of more indigenous chronic tests for use in DTA and derivation of guideline values is required.

Selection of distribution type (log-normal, Burr Type III or Pareto) had only a small effect (typically 25%) on the derived PC95 values. There was no consistent difference between PC95 estimates of the two distribution types, i.e. PC95 values estimated by the Burr Type III or Reciprocal Pareto distributions were not consistently higher or lower than PC95 values estimated by the log-normal distribution. The influence of the selection of ACR and

inclusion of larval development tests on the SSD and PC95 values was assessed by three additional scenarios. Increasing the ACR from 5 to 10 (Additional Scenario 1), increased the TV by between 15% and 50%, contrary to what would be expected as increasing the ACR would decrease the individual values in the NOEC dataset used to generate the SSDs. When the larval development tests were included as acute tests (Additional Scenario 2) and an ACR of 5 applied to the acute data, the resulting PC95 derived by BurrliOZ decreased by 5%, however, the distribution altered from a Pareto distribution to a Burr Type III distribution. In contrast the PC95 calculated by ETX decreased by 40%. When both input parameters were altered in the most conservative estimates, i.e. applying an ACR of 10 and including larval development tests as acute tests (Additional Scenario 3), the TVs decreased by ~3 fold irrespective of which method was used. The manipulation of input data to the SSD, through selection of the ACR and classification of sub-chronic larval development tests as either acute or chronic tests, had a considerably greater effect on the resulting PC95, than the choice of distribution type. This finding is similar to the observations of (Duboudin *et al.* 2004). The ACR of 5 derived for narcotic contaminants in other studies (Di Toro *et al.* 2000; De Zwart 2002) is considered more accurate than the arbitrary default ACR of 10 provided in ANZECC and ARMCANZ WQGs (2000). Since the release of the Australian WQGs in 2000 the consensus seems to have been reached (USEPA 2002; Stauber *et al.* 2004; Warne 2008) that ELS testing is a sub-chronic exposure and that the data can be considered as chronic for the derivation of WQGs. Thus, the ELS data for the oyster and sea urchin should be used as chronic toxicity data to calculate site-specific PC95 values.

The dataset used for the derivation of the SSDs in the current study was relatively small with only 5 observations and the influence of selection of ACR and classification of test type on this small number of observations was observed to result in up to a threefold difference in the resulting PC95 values. This number does, however, meet the requirements of Australia and New Zealand (Kefford *et al.* 2005). Although this small dataset meets the minimum sample requirements, it does make the derived PC95 values more sensitive to transformation of the dataset, i.e. by application of ACRs or inclusion of tests as either acute of chronic tests. A review of existing extensive datasets for pesticides

suggested that least 30 data points should be used to minimise variability in derived SSDs, with this number varying between 15 and 55 (Newman *et al.* 2000a). The same review noted that the inability to meet the required sample size to minimise variability does not make the approach invalid, merely results and interpretation should be treated with caution (Newman *et al.* 2000a). Between 19 and 23 data points, derived using QSARs, were used in the derivation of the ANZECC and ARMCANZ (2000) TVs for VCHs. Testing of such a large number of species, however, is a large undertaking and probably not appropriate or warranted for derivation of site-specific guideline values.

Although other researchers have assessed the toxicity of contaminated groundwater (Kszoz *et al.* 2003; Zolezzi *et al.* 2005), neither of these studies derived a risk-based, site-specific guideline for contaminated groundwater using a SSD. The regulatory guidance in Australia supports the derivation of site-specific guidelines (NEPC 1999; ANZECC and ARMCANZ 2000). The similarity between regional SSDs and site-specific SSDs, as assessed by Bossuyt *et al.* (2005), is consistent with the conceptual underpinning and supports derivation of site-specific guidelines using SSDs. The SSD approach enables managers or regulatory authorities to select a number of risk-based site-specific TVs which could include PC99, PC90, PC95 or PC80 values, i.e. the levels of protection provided in the Australian and New Zealand WQGs (ANZECC and ARMCANZ 2000) depending on the level of risk acceptable to regulatory authorities or as interim remedial targets, based on the condition of the site. The approach presented in the current study would also be suitable for incorporation into future probabilistic ecological risk assessment.

CONCLUSIONS

It is recommended that the SSD and PC95 value of 830 µg/L total volatile chlorinated hydrocarbons derived using the log-normal distribution be adopted as the site-specific guideline. The log-normal distribution was a marginally better fit than the Reciprocal Pareto distribution. In addition, the Reciprocal Pareto distribution is a finite threshold model that does not accurately reflect the toxicity of the contaminants in this study.

Choice of the type of distribution had a smaller effect (~25%) on derived PC95 values than classifying larval early life stage development tests as acute or chronic tests and the selection of acute to chronic ratios of 5 or 10. Through deriving PC95 values in different scenarios, differences of up to threefold were identified. The small number of indigenous species available for toxicity testing and the even smaller number of species for which chronic tests are available, greatly affects the choice of tests and possibly, the derived distributions and guideline values. Therefore, continued development of chronic indigenous test organisms is recommended.

The current study demonstrated that a site-specific, risk-based guideline for a complex mixture of VCHs may be derived using an SSD derived from DTA on a battery of indigenous test species.

PAPER 4

SITE-SPECIFIC PROBABILISTIC ECOLOGICAL RISK ASSESSMENT OF A VOLATILE CHLORINATED HYDROCARBON CONTAMINATED TIDAL ESTUARY

ABSTRACT

Investigation of groundwater at an industrial facility indicated that groundwater contaminated with volatile chlorinated hydrocarbons (VCHs) discharged via stormwater drains to Penrhyn Estuary, an intertidal embayment of Botany Bay, NSW. A screening level hazard assessment of surface water in Penrhyn Estuary identified that the VCHs posed a potential to marine organisms. Given the known limitations of hazard assessment, the current study conducted a higher tier, quantitative probabilistic risk assessment using the joint probability curve (JPC) method that uses probability distributions to account for variability in both exposure and toxicity profiles to quantify risk (δ).

Risk was assessed for 24 difference scenarios. The exposure scenarios were four areas of the estuary for exposures based on low tide, high tide and a combination of low and high tide concentration data. Toxicity scenarios were based on data for no observed effect concentrations (NOEC) to assess potential for possible adverse effects to organisms and effect concentration to 50% of test organisms (EC50), to assess the risk of strong adverse effects to the ecological community. Risk was consistently greater at low tide than at high tide and varied throughout the tidal cycle. The exposure scenario using data combined from both tides was considered the most accurate representation of the ecological risk in the estuary. The spatial distributions of risk were similar using both NOEC and EC50 data. When assessing risk using data across both tides, the greatest risk was identified in the Springvale Tributary (δ=25%) – closest to the Source Area, followed by the Inner Estuary (δ=4%) and the Floodvale Tributary (δ=2%), with the lowest risk in the Outer Estuary (δ=0.1%) – furthest from the source area. The JPC methodology also provided an indication of the type of exposure.

INTRODUCTION

Estuaries are often receiving ecosystems for contaminants from numerous sources, including discharge of stormwater and groundwater containing various organic and inorganic contaminants (Bickford *et al.* 1999; Burton *et al.* 2002; Lakatos *et al.* 2003; Brown and Ferris 2004). Groundwater contamination by chlorinated hydrocarbons and industrial solvents is widespread (USEPA 1990; Zolezzi *et al.* 2005) and a screening level hazard assessment identified an unacceptable hazard posed by volatile chlorinated hydrocarbon (VCH) contamination of Penrhyn Estuary in Sydney, Australia (Hunt *et al.* 2007). This hazard assessment identified a number of limitations including, the use of the hazard quotient (HQ) approach; use of *low reliability* water quality trigger values (TVs) and highly variable exposure. The need for direct toxicity testing of the complex mixture of VCHs and for more informative, higher tier assessment of the ecological risk was recognised (Hunt *et al.* 2007). The HQ approach itself was identified as a limitation as it used point estimates for exposure (i.e. using mean concentrations) and toxicity (i.e. using TVs for individual VCHs) and ignored the variability inherent in these parameters and lacked a measure of risk (probability). Although the HQ approach is the most common risk characterisation methodology (Calabrese and Baldwin 1993), it is only useful for screening level assessments as its reliance on point estimates does not consider variability in exposure (Solomon and Sibley 2002) or the relationship between concentration and effects (Solomon and Takacs 2002) and therefore, HQs cannot estimate the magnitude of risk (Sorenson *et al.* 2004). Quotients based on single point estimates are not defensible for higher tier assessments of ecological risk as much of the data are disregarded and the risk is not estimated in probabilistic terms (Bartell 1996).

The general ecological risk assessment (ERA) framework provided in the United States (USEPA 1998) has been adopted in Australia (NEPC 1999). The ERA paradigm presented by Suter (1993) included several phases including: problem formulation; exposure assessment; toxicity assessment and risk characterisation. The final elements of the paradigm comprised risk communication and management. Risk assessment process is frequently a tiered approach with lower tiers generally more conservative with simple analyses and conservative assumptions that overestimate risk. Potential risk identified at

lower tiers triggers assessment at higher tiers, which may be site-specific with more detailed, realistic characterisations with less conservative assumptions as in the current study (Solomon and Takacs 2002; Burgman 2005). A risk based approach was adopted in the national water quality guidelines (ANZECC and ARMCANZ 2000), despite ERA for aquatic ecosystems being relatively new in Australia. Local ERAs have been undertaken for river management (Hart *et al.* 2001; Hart *et al.* 2006), pesticides (Muschal and Warne 2003a), herbicides (van Dam *et al.* 2004), salinity (Hart *et al.* 2003), discharges of sewage in the Sydney Region (Bickford *et al.* 1999) and mining (Brown and Ferris 2004).

Risk (δ) is most simply defined as the likelihood of an adverse event occurring, or in toxicological terms, as the product of the likelihood (exposure) and the consequence (toxicity) (Hart *et al.* 2006). Exposure characterisation should characterise the spatial or temporal distribution of the stressor and co-occurrence with ecological endpoints. The effects characterisation should identify and quantify the effects of the stressor and evaluate cause and effect relationships to the extent possible (Hart *et al.* 2001). Exposure distributions can be derived from modelling or monitoring programs (ECOFRAM 1999). Modelling is a suitable method for regional scale assessments, particularly for predictive assessments of herbicides or pesticides (e.g. (Ritter *et al.* 2000; Solomon *et al.* 2000), however, for site-specific studies, data are typically derived from monitoring programs (Poletika *et al.* 2002), as is the case with the current study. Toxicity distributions can be derived from published toxicity data (Brix *et al.* 2001; van Sprang *et al.* 2004), or as in the current study, site-specific toxicity data.

Probabilistic ERAs (PERA), which qualify and quantify ecological risks using exposure and effects probability distributions, are a considerable improvement on the HQ approach (Solomon 1996; ECOFRAM 1999). Conversion of a hazard assessment to a risk assessment requires a probabilistic element to determine the *likelihood* of a hazard having an effect, which point estimates cannot provide (Solomon *et al.* 2000; Burgman 2005). The Probabilistic ERAs approach is currently being implemented by USEPA (USEPA 2000b) is most commonly used in predictive risk assessments for assessment of the potential for

adverse effects resulting from use of new chemicals, e.g. pesticides and pharmaceuticals (Solomon *et al.* 2000; Poletika *et al.* 2002; Reiss *et al.* 2002; Cunningham *et al.* 2004; Hela *et al.* 2005; Capdevielle *et al.* 2008). Probabilistic techniques have been used to assess surface water contamination (Hall *et al.* 1998; Brix *et al.* 2001; van Sprang *et al.* 2004; Bossuyt *et al.* 2005), however, these studies have been undertaken at regional scales and commonly do not include probabilistic elements for both toxicity and exposure. As most Probabilistic ERAs are undertaken for chemical registration, risk assessment of site contamination can be improved through adoption of techniques currently utilised in PERAs for chemical registration. PERAs were developed because worst-case scenarios typically overestimate exposure, are overly conservative and unrealistic, whilst probabilistic methods are more realistic and give more information to managers (Solomon and Takacs 2002). Although more data are generally required, a key advantage of PERAs is that use of distributions for exposure and toxicity allows quantitative estimation of risk (Solomon and Takacs 2002) and can incorporate variability and uncertainty into risk estimates (Roberts 1999).

In Probabilistic ERA, estimation of risk is described as being proportional to the degree of overlap of the distributions (Solomon *et al.* 2000) and when exposure and toxicity data are plotted on the same axes, the extent of overlap between the two distributions indicates the probability of exceeding an exposure concentration associated with a particular probability of effects or concentration which is accepted to protect and preserve ecosystem structure and function (Solomon 1996; Solomon *et al.* 2000; Solomon and Takacs 2002). One method of displaying risk is through the use of joint probability curves (JPCs) for which the area under the JPC has been shown to be mathematically equivalent to the overlap of the exposure and toxicity curves (Aldenberg *et al.* 2002; van Straalen 2002) (see Methodology – Risk Characterisation).

The objective of the current study was to undertake a probabilistic ERA at a site-specific scale using indigenous Australian species and assessing the risk posed by discharge of contaminated groundwater to an estuary using probability distributions for both toxicity and exposure using the JPC methodology. The current ERA was also undertaken to

address the limitations of the screening level hazard assessment (Hunt *et al.* 2007) and provide a higher tier assessment for a more accurate estimation of ecological risk.

PROBLEM FORMULATION

A problem formulation phase was originally provided in the screening level hazard assessment (Hunt *et al.* 2007), however, a summary is provided. Penrhyn Estuary is a small (10 ha) tidal embayment located approximately 10 km south of the Sydney central business district on the northern shoreline of Botany Bay, New South Wales (NSW), Australia (Figure 1). Land use in the 320 ha catchment includes residential, commercial and both light and heavy industrial. The embayment is inundated at high tide with water covering an area of approximately 4.0 ha. At low tide, mudflats are exposed and the inundated area is approximately 0.4 ha. It was originally devoid of vegetation when it was formed in the late 1970s using sandy dredge spoil from development of the adjacent port. However, today it supports a variety of flora species, including mangroves, saltmarsh species and dune vegetation and also attracts wading shorebirds which forage on the mudflats at low tide. The fauna are typical of those found in south eastern Australian marine and estuarine environments (Edgar 1997).

Shallow (i.e. <3m below ground surface) groundwater with at least 14 VCHs discharges into two drains – Springvale Drain and Floodvale Drain – and flows into the estuary (AGEE and Woodward-Clyde 1990; Woodward-Clyde 1996). This contamination has been relatively continuous since at least the 1990s. Contamination of surface water in the estuary is complex and concentrations of VCHs are a product of tidal regime, rainfall and source contribution (URS 2005). For the purposes of the current risk assessment, the estuary was divided into 4 areas: the Springvale Tributary; the Floodvale Tributary; the inner estuary; and the outer estuary (Figure 1). The inner estuary discharges to the outer estuary which discharges to Botany Bay. A typical salinity gradient exists in the estuary, from the fresh discharge in the upper reaches, to the saline inflow from Botany Bay.

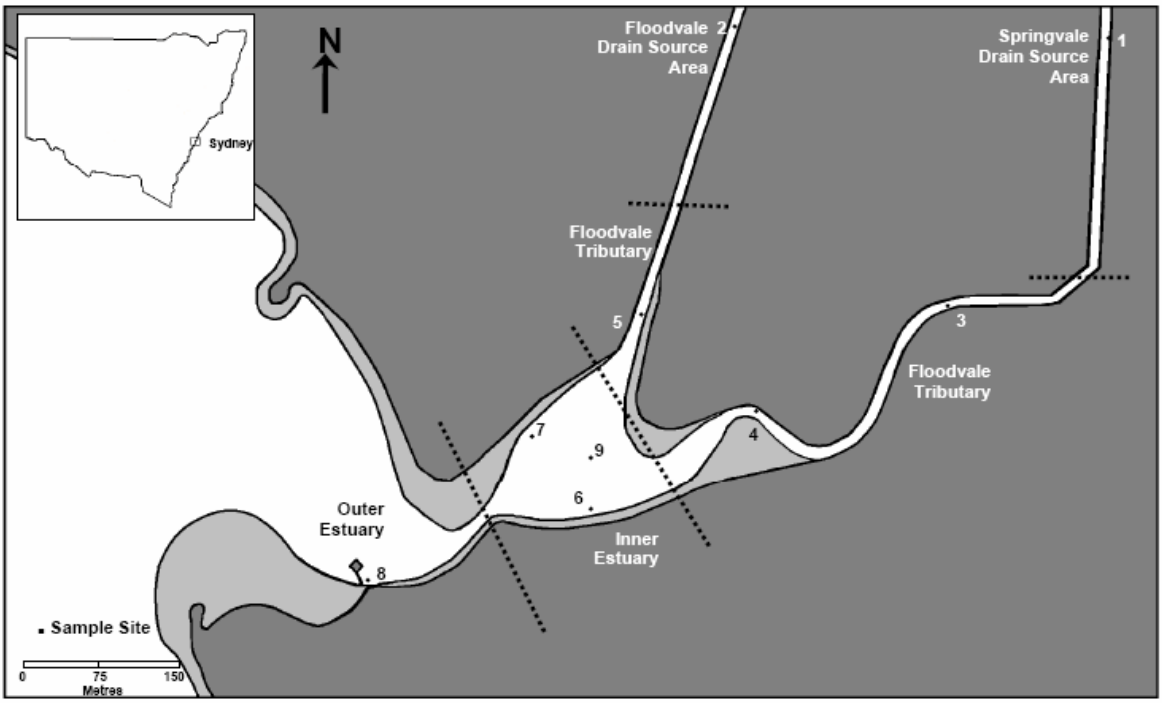


Figure 1. Location plan of Penrhyn Estuary, Sydney, Australia indicating Source Areas in a) Springvale Drain (SVD), b) Floodvale Drain (FVD) and Penrhyn Estuary including c) Floodvale Tributary (FVT), d) Springvale Tributary (SVT), e) Inner Estuary (IE) and f) Outer Estuary (OE). Dashed lines denote boundaries between zones within the estuary.

The VCHs in the current study are characterised by low boiling points, high vapour pressures and high water solubility. These chemicals typically have octanol water partition coefficients (K_{ow}) of <3, indicating high water solubility, low potential for bioaccumulation; with a low tendency to bind to organic carbon, sediment, suspended particulate matter or dissolved organic carbon (Carey *et al.* 1998). Direct exposure to VCHs in the water column is therefore likely to be the primary source of uptake, with food and sediment ingestion probably being a minor component of uptake (Hunt *et al.* 2007). The predicted equilibrium distribution of VCHs is approximately 99% distributed in the atmosphere and 1% partitioning to water (Carey *et al.* 1998). The key process reducing concentrations of VCHs in the estuary is dilution by inflowing seawater from Botany Bay (URS 2005). The VCHs in the present study act under the narcotic mode of action (McCarty and Mackay 1993b; Carey *et al.* 1998).

The previous screening level hazard assessment (Hunt *et al.* 2007) concluded that there was a greater hazard at low tide than high tide throughout the estuary; there was a greater hazard in the upper estuary than in the Inner Estuary or the Outer Estuary; the hazard was highly variable both spatially and temporally; and the hazard posed by the contaminant mixture was greater than that posed by individual contaminants.

The assessment hypothesis for the current risk assessment is that exposure to VCHs would result in adverse effects to the aquatic ecological community present in Penrhyn Estuary and Botany Bay. The potential exposure of the organisms in the ecosystem is modelled by the distribution of measured exposure concentrations of VCHs, whilst the ecotoxicological effects are modelled by the site-specific SSD derived from direct toxicity assessment (Hunt *et al.* 2009b). Implicit in the assessment methodology is that the SSD and protection of a percentage of species will result in protection of the structure and function of the ecosystem. Following derivation of distributions for exposure and toxicity, the potential ecological risk was quantified by measurement of overlap between these two distributions.

METHODOLOGY

EXPOSURE ASSESSMENT

To characterise exposure to VCHs in surface waters in the estuary, data from two surface water monitoring programs were pooled to provide a combined dataset (Hunt *et al.* 2007). Seven sites were selected to characterise various zones in the study area with two sites located in Springvale Tributary (SVT), one site in the Floodvale Tributary (FVT), three sites in the inner estuary (IE) and one in the outer estuary (OE)(Figure 1). Samples were collected from the concrete-lined stormwater drain at the head of Springvale Drain and Floodvale Drain in the source area. However, as these drains do not constitute ecosystems, they have not been assessed for ecological risk; nevertheless they were included for comparative purposes and source characterisation.

Sampling of estuarine water from the seven sites and subsequent analysis for VCHs was undertaken over a one-year period (in 2004 and 2005) in two monitoring programs to characterise exposure to concentrations of VCHs in surface water in the estuary. In the first program, samples were collected every three months to assess variability in concentrations of VCHs in the estuary over the year (URS 2004b), whereas in the second program samples were collected over one month to assess short-term variability (URS 2005). It is important to match the variability of the sampling program to the variability of the system being measured (Solomon and Takacs 2002) and as the estuary is tidal, samples were generally collected at high and low tides, representing the lowest and highest concentrations of VCHs, respectively. One sampling round was common to both programs and samples were not collected from all locations on all occasions. Data were compiled from sites within the four areas comprising; Springvale Tributary (n = 22); the Floodvale Tributary (n = 13); the Inner Estuary (n = 22); and the Outer Estuary (n = 13). The number of samples available for assessment using both tides in these areas was n = 44 samples (in each of Springvale Tributary and the Inner Estuary) and n = 26 samples (in each of Floodvale Tributary and the Outer Estuary), respectively. Samples for both programs were collected during periods of wet and dry weather to characterise temporal variability of VCHs. Three exposure scenarios were presented in Hunt *et al.* (2007) and adopted in the

current study; Scenario 1 – the aqueous concentrations measured across high and low tides, Scenario 2 – the aqueous concentrations measured at high tide, and Scenario 3 – the aqueous concentrations measured at low tide.

Surface water samples were collected in 40 mL glass vials with airtight Teflon™ lined lids with zero headspace. Samples were immediately preserved in the field with hydrochloric acid and immediately stored <4°C (Hunt *et al.* 2007). Samples were extracted using purge and trap methodology (USEPA 5030B) and analysed by Gas Chromatography Mass Spectrometry (GC/MS) utilising a modification of the USEPA Method 8260B for volatile organic compounds (USEPA 1996c) and the same method used to quantify VCHs in the DTA (Hunt *et al.* 2009b). The limit of reporting was 1 µg/L for all analytes with the exception of vinyl chloride (10 µg/L). Hunt *et al.* (2007; 2009b) reported that quality control evaluations were undertaken on each of the sample batches and no analytes were detected in the method blanks. Recoveries for laboratory control samples and matrix spikes were between 80 to 120%, and within the acceptable criteria. It was further reported that differences between primary and duplicate samples were generally <25%, which was identified as typical of the variability observed between duplicate samples for these contaminants at this laboratory and was considered acceptable (Hunt *et al.* 2007).

Values that are less than the limit of reporting (LOR) are commonly encountered in monitoring programs and risk assessments, as is the case in the present study, where the true concentration in the sample may be somewhere between zero and the LOR (Warren Hicks *et al.* 2002). The four options available to deal with results less than the LOR are: to include the concentrations as zero values which would underestimate exposure and risk; to include the concentrations as half the LOR; to include the concentrations as equal to the LOR, which is likely to overestimate exposure and subsequent risk (Warren Hicks *et al.* 2002); or assume that the data <LOR have a distribution, log-normal or the same as the data >LOR. In the present study, the moderately conservative approach of including data as half the LOR was adopted. As environmental data are often log-normally distributed (Gilbert 1987), distributions of each of the exposure datasets (Scenarios 1 to 3) were

assessed for log-normality using the Anderson-Darling test to assess their suitability for use in the JPC method.

EFFECTS ASSESSMENT

The effects assessment component of aquatic ERAs is commonly undertaken using conservative point estimates of effects, such as water quality guidelines (WQGs) (USEPA 1998; NEPC 1999). The screening level hazard assessment conducted previously for the same sites (Hunt *et al.* 2007) used 95% TVs provided for VCHs in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (ANZECC and ARMCANZ 2000), and new TVs were derived where they were not available (Hunt *et al.* 2007). These TVs aim to protect 95% of species and are equivalent to a hazardous concentration to 5% of species (HC5) that are commonly used in Europe. As TVs were exceeded and a complex mixture of potentially additive contaminants was present, direct toxicity assessment (DTA) was required, in accordance with the ANZECC and ARMCANZ (2000) decision tree (Hunt *et al.* 2007). There is very little toxicity data for VCHs, as evident by the fact that the Australian and New Zealand TVs for these compounds are based on QSAR derived estimates of toxicity (ANZECC and ARMCANZ, 2000). In addition, the risk characterisation will be conducted using a SSD method. Five organisms belonging to four different taxa, and therefore, meeting the minimum data requirements to use an SSD in the Australian and New Zealand WQGs (ANZECC and ARMCANZ, 2000), were selected to characterise the exposure of organisms in Penrhyn Estuary.

As the toxicity tests will be conducted using surface water samples from Penrhyn Estuary it is a form of direct toxicity assessment (DTA) or whole effluent toxicity (WET) testing. Criteria to be used in selecting test species for DTA include: having regional relevance; having a wide geographical distribution; having economic importance; being sensitivity to the contaminants; having a sensitive life stage; and belonging to different taxonomic groups and trophic levels (ANZECC and ARMCANZ 2000; van Dam and Chapman 2001). The battery of test organisms selected in the current study were the microalga *Nitzschia closterium*, the sea urchin *Heliocidaris tuberculata*; the Sydney Rock oyster

Saccostrea commercialis; the amphipod *Allorchestes compressa*; and the polychaete *Diopatra dentata*. These organisms meet the above criteria (Hunt *et al.* 2009b).

Toxicity tests included; a 72 h algal population growth bioassay (*N.closterium*); a 72 h sea urchin larval development bioassay (*H.tuberculata*); a 72 h oyster larval development bioassay (*S.commercialis*); amphipod 96 h survival bioassay (*A.compressa*); and a 96 h juvenile polychaete (*D.dentata*) survival bioassay. Toxicity testing was undertaken in sealed containers to prevent loss of volatile contaminants and potential underestimation of toxicity. Detailed test methods are provided in Hunt *et al.* (2009a). Of the above tests, the algal test is a chronic test and the larval development tests (i.e. for the sea urchin and oyster) are sub-chronic tests. The larval development tests were treated as chronic tests in the derivation of the SSD as early life stage testing such as this can be considered chronic in the derivation of water quality guidelines (USEPA 2002; Warne 2008). The amphipod and polychaete tests, however, are acute tests and application of an acute to chronic ratio (ACR) was required to convert EC50 data to chronic NOEC data, before use in deriving the SSD. An ACR of 5 was selected as ACRs for non-polar narcotic chemicals have generally been reported to be close to 5, with estimations of 4.5 ± 2.5 (McGrath *et al.* 2004) and 5.09 ± 0.95 (Di Toro *et al.* 2000).

The probability distribution for the toxicity was the SSD derived for the contaminated groundwater derived from the DTA presented in Hunt *et al.* (2009b). Hunt *et al.* (2009b) evaluated Reciprocal Pareto and log-normal distributions for the derivation of the site-specific SSD and concluded that as the Reciprocal Pareto distribution represented a finite threshold model that was less applicable to the toxic mode of action of VCHs than the continuous log-normal distribution and as the log-normal distribution was a marginally better fit, it should be adopted as the site-specific SSD. The log-normal curve has been used extensively in the derivation of SSDs (Aldenberg *et al.* 2002; Hanson and Solomon 2002) and there is extensive information available on the application of log-normal distributions to JPCs (Solomon and Takacs 2002). Solomon and Takacs (2002) and ECOFRAM (1999) suggested grouping similar organisms and deriving separate SSDs for

groups of species, however, the current study was undertaken in accordance with ANZECC and ARMCANZ (2000), which recommends deriving one SSD with all organisms included in one dataset. The requirement for derivation of separate SSDs is more likely to reflect differing sensitivities to contaminants with a specific mode of action (i.e. pesticides), unlike the narcotic contaminants in the present study, which have been shown to exhibit low variation in species sensitivity with levels of toxicity predictable on the basis of hydrophobicity (Vall *et al.* 1997).

Two toxicity scenarios were evaluated in this study. In the first toxicity scenario, an SSD was derived using NOEC data, and is considered to represent mild ecological risk. In the second toxicity scenario, an SSD was derived using EC50 data and is considered to represent the risk of significant adverse effects to ecological receptors. It has been suggested that the EC/LC50 metric may be an indicator of actual effects occurring in the ecosystem (Solomon *et al.* 2001). It has also been suggested that this may be a more useful measure of the effect to the population as compensatory mechanisms may occur when other more conservative metrics are used (Daniels and Allen 1981; Day and Kaushik 1987). In other studies, ecologically significant effects have generally been observed at concentrations exceeding the EC25 level of laboratory based distributions (Hall and Giddings 2000; Giddings *et al.* 2001).

The conceptual model of ecological risk underlying these scenarios is that as an increasing number of species are affected, the number of organisms available to fulfil the roles required for ecosystem structure and functioning would decrease. Having concentrations that exceeded the outcomes from these two scenarios, would be associated with different ecological outcomes, with the first scenario predicting a lack of protection and the second scenario predicting likely adverse effects.

RISK CHARACTERISATION

As an SSD (with proportion of species affected and concentration of contaminant) and exposure probability plot (with concentration of contaminant and probability) have a

common axis, i.e. the concentration of contaminant, the axes can be rationalised into a single plot, the JPC with two axes – probability and proportion of species affected. The shape of the JPC can be also be used to define acceptable or unacceptable ecological risks, providing an indication of the type and duration of exposure. The area under the curve, the quantified risk (δ) is a product of the shape of the curve and has been shown to be mathematically equivalent to the overlap of the exposure and toxicity curves (Aldenberg *et al.* 2002; van Straalen 2002). These two measures, the shape and quantified risk (δ), are complementary measures, as δ alone does not capture all aspects of the shape of the curve (Verdonck *et al.* 2003). The summary statistic (δ), as in all risk assessments, equates a high probability/low damage event with a low probability/high damage event (van Straalen 2002). These scenarios have different ecological implications and the shape of the curve, importantly, allows consideration of the type of risk alongside total risk. Verdonck *et al.* (2003) demonstrated that the shape of the JPC could be used to differentiate between risk profiles where 50% of organisms may die nearly 100% of the time, to a scenario where 100% of organisms may die 50% of the time, each with δ = 50%. Quantification of risk and the shape of the curve are conceptually, readily understood and therefore, support risk communication, the final phase of risk assessment.

The ETX™ program (van Vlaardingen *et al.* 2004), which estimates log-normal curves for each of exposure and toxicity distributions, was used to generate a JPC curve and δ. This was undertaken for three tidal exposures (i.e. data across both tides and high and low tides alone), for two toxicity scenarios (i.e. using NOEC and EC50 data) for each of the four areas (Springvale and Floodvale Tributaries and the Inner and Outer Estuaries), resulting in 24 values. Where the standard deviation of the exposure data was too large for ETX™ to calculate δ, it was estimated manually from the JPC.

In the current study, the adopted threshold of 'acceptable risk' (i.e. δ) was 5%. This magnitude of risk is inherent in the risk-based framework provided in the ANZECC and ARMCANZ (2000) Guidelines for Fresh and Marine Water Quality. In ANZECC and ARMCANZ (2000), 95% TVs are recommended for adoption for slightly to moderately

disturbed ecosystems, such as Penrhyn Estuary. A 95% TV represents protection of 95% of species. A δ value of 5% is equivalent to 5% of species being adversely affected 100% of the time, although not equivalent, this is acceptable given the adoption of 95% TVs, i.e. as 5% of species or less are affected, no further action or assessment is triggered.

RESULTS AND DISCUSSION

EXPOSURE ASSESSMENT

The total number of samples available to characterise surface water in the estuary varied from a minimum of 13 samples to a maximum of 44 samples. Mean concentrations of VCHs in the Source Area varied from 22 035 µg/L (Springvale Drain) to 1 420 µg/L (Floodvale Drain). Mean concentrations of VCHs in the estuary varied from a minimum of 21 µg/L at high tide in the Outer Estuary to a maximum of 3 984 µg/L at low tide in the Springvale Tributary (Table 1).

Concentrations of VCHs were generally greatest at low tide > both tides > high tide (Hunt *et al. 2007*). Concentrations generally decreased downstream with Springvale Tributary > Inner Estuary > Floodvale Tributary > Outer Estuary. The highest concentrations of VCHs were observed in Springvale Drain and the upper estuary whereas, the lowest concentrations in the Outer Estuary where surface water discharges to Botany Bay. Concentrations in all areas of the estuary were highly variable due to fluctuations in the tidal cycle; tidal height; and rainfall (Hunt *et al.* (2007).

The distribution of each of the exposure scenarios was tested for normality using the Anderson-Darling test. Exposures were log-normally distributed for 75% of scenarios, with the exception of three exposure scenarios: in Springvale Tributary across both tides: and in the Outer Estuary, across both tides and when high tide was assessed alone. The Outer Estuary had the lowest number of VCH detections (~35%), with the majority of samples being <LOR, which may contribute to the lack of normality of the distribution. The implication of the exposure data failing the Anderson-Darling test for log-normality is unclear and the robustness of the assumption that the exposure data fits a log-normal distribution has not been fully evaluated. It has been suggested by Newman *et al.* (2000b) that violation of this assumption, whilst undesirable, may have little effect on the resulting interpretation of risk.

Table 1. Exposure data - concentrations of volatile chlorinated hydrocarbons (in µg/L) in surface water in Penrhyn Estuary.

	Springvale Drain		Floodvale Drain		Springvale Tributary		Floodvale Tributary		Inner Estuary		Outer Estuary	
	Mean	**St.Dev**	**Mean**	**St.Dev**	**Mean**	**St.Dev**	**Mean**	**St.Dev**	**Mean**	**St.Dev**	**Mean**	**St.Dev**
Both Tides	22035.9	18865.2	1419.8	685.3	2779.5	4474.2	400.3	314.5	611.7	1302.6	85.0	162.4
High Tide	--	--	--	--	1575.1	1984.3	194.7	136.1	78.0	81.6	21.0	23.9
Low Tide	--	--	--	--	3983.9	5832.1	606.0	309.6	1145.4	1694.3	149.0	213.3

- Indicates that no data is available as the location is not tidal

EFFECTS ASSESSMENT

Metrics, including NOEC, LOEC and EC50 were calculated for the DTA testing as concentrations of total VCHs (Table 2). NOECs varied from 1.11 mg/L (urchin larval development test) to 45.5 mg/L (amphipod survival test), whereas EC50 values varied from 3.77 mg/L (urchin larval development test) to >45.5 mg/L (amphipod survival test).

Toxicity data were normally distributed for both toxicity scenarios assessed using the Anderson-Darling test ($p<0.05$). SSDs were derived for each of the NOEC and EC50 toxicity scenarios (Figures 2a and 2b). HC5 values for the scenarios varied from 830 µg/L (NOEC) to 1,520 µg/L (EC50).

RISK CHARACTERISATION

The JPC approach was used for the three tidal exposures (i.e. both, high and low tides) for the two toxicity scenarios (i.e. NOEC and EC50) and for each of the four areas (Springvale and Floodvale Tributaries and the Inner and Outer Estuaries), resulting in a total of 24 assessments for risk (δ) (Tables 3a and 3b). Risk (δ) was estimated manually from the JPC for two of the twelve EC50 scenarios and one of the NOEC scenarios.

In the NOEC toxicity scenario, risk values (δ) varied from a minimum of 0.00% (in the Outer Estuary at high tide) to a maximum of 36% (in Springvale Tributary at low tide) (Table 3a). In the EC50 scenario, risk values (δ) varied from a minimum of 0.00% (in five of the twelve scenarios) to a maximum of 14% (in Springvale Tributary at low tide) (Table 3b). In the Source Areas, the risk (δ) in the NOEC scenario varied from 84% in Springvale Drain to 16% in Floodvale Drain. In the EC50 Scenario, the risk (δ) varied from 55% in Springvale Drain to 4% in Floodvale Drain (Table 3b).

Table 2. Summary of NOEC, LOEC and EC50 data for bioassays exposed to VCH groundwater (total VCHs in mg/L).

Toxicity Metric	Alga	Urchin	Oyster	Polychaete	Amphipod
NOEC	2.3	1.11	4.98	29.88	45.5
LOEC	4.98	2.3	10.31	45.5	45.5
EC50	4.1	3.77	9.79	32.08	>45.50

Table 3. Ecological risk in the four locations across the three tidal (exposure) scenarios for both NOEC and EC50 (toxicity) scenarios.

Table 3a)

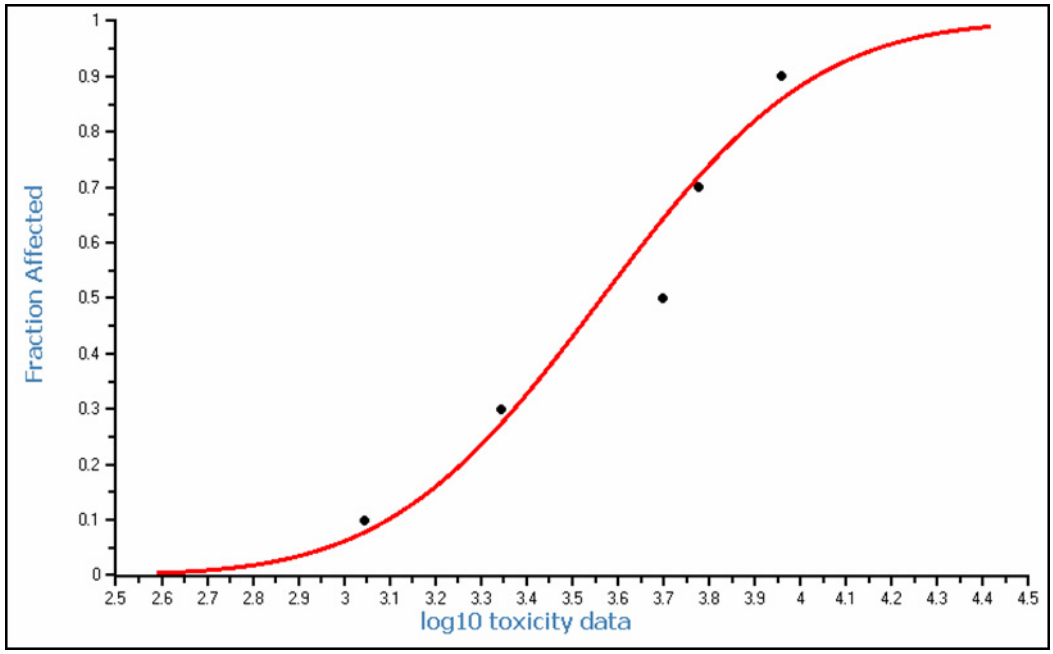
NOEC Scenario	Springvale Drain Source Area	Floodvale Drain Source Area	Springvale Tributary	Floodvale Tributary	Inner Estuary	Outer Estuary
Both	**83.8**	**15.9**	25.0	2.6	4.1	0.1
High	--	--	*16.0*	0.1	0.1	0.0
Low	--	--	35.4	2.3	9.3	0.8

Table 3b)

EC50 Scenario	Springvale Drain Source Area	Floodvale Drain Source Area	Springvale Tributary	Floodvale Tributary	Inner Estuary	Outer Estuary
Both	**55.3**	**4.2**	*11.3*	*0.7*	*1.5*	0.0
High	--	--	*7.0*	*0.1*	*0.0*	0.0
Low	--	--	*13.9*	0.7	*3.3*	0.0

-- no risk values were derived as the location is not tidal.

a) NOEC species sensitivity distribution



b) EC50 species sensitivity distribution

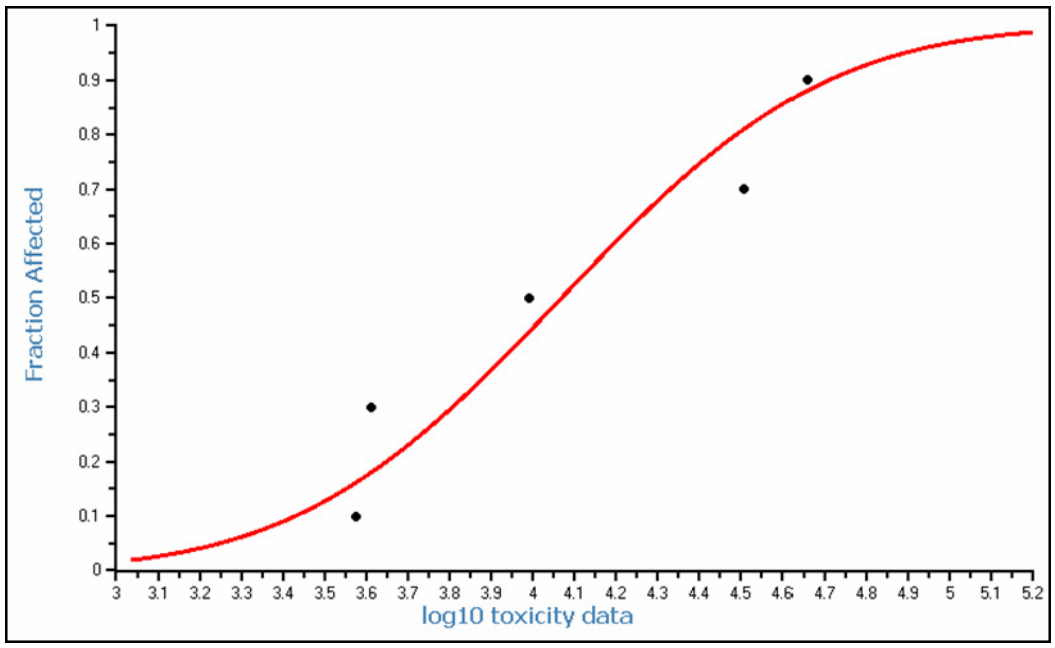


Figure 2a) and 2b). Species sensitivity distributions for a) NOEC and b) EC50 scenarios.

Similar patterns were observed in the risk characterisation for the NOEC and EC50 scenarios. Of the twelve NOEC scenarios, risk (δ) was <5% in approximately 66% of the results, between 5% and 10% in approximately 8% of the scenarios and >10% in 25% of the scenarios. For the EC50 scenario, the risk (δ) was less than 5% in approximately 75% of the results, between 5% and 10% in approximately 8% of the scenarios and greater than 10% in 17% of the scenarios.

SPATIAL INTERPRETATION OF RISK

Spatially, the pattern of risk was similar between the NOEC and EC50 Scenarios. Risk typically decreased in the following order SVT >> IE > FVT > OE. This was the case for both NOEC and EC50 toxicity scenarios in the three tidal scenarios with the exception of the high tide NOEC Scenario, where the risk was marginally greater in FVT than the IE. The greatest risk was recorded for Springvale Tributary (mean $\delta = 19.7$, $n = 6$) (Tables 3a) followed in order of decreasing risk by the Inner Estuary (mean $\delta = 2.8$, $n = 6$), the Floodvale Tributary (mean $\delta = 0.91$, $n = 6$) and the Outer Estuary (mean $\delta = 0.15$, $n = 6$).

As interpretations of risk associated with each of the different toxicity distributions and with each of the exposure distributions are similar in nature and only differed in magnitude, the detailed spatial interpretation of risk will only be provided below for one scenario – the NOEC toxicity data and both high and low tide data.

Although not representing ecosystems requiring protection, the Springvale Drain and Floodvale Drains were included in the assessment for the purposes of characterising risk at the source area. The source area is not tidal and therefore, only one exposure scenario was assessed. The risk in the Springvale Drain was 84% and in Floodvale Drain was 15% (Table 3a) with the JPCs reflecting the differing inverse ecological risk profiles (Figures 3a and 3b). The JPC for Springvale Drain (Figure 3a) represents an undesirable curve shape associated with high environmental risk, with most species being affected the majority of the time, whereas the JPC for Floodvale Drain (Figure 3b) represents a more desirable

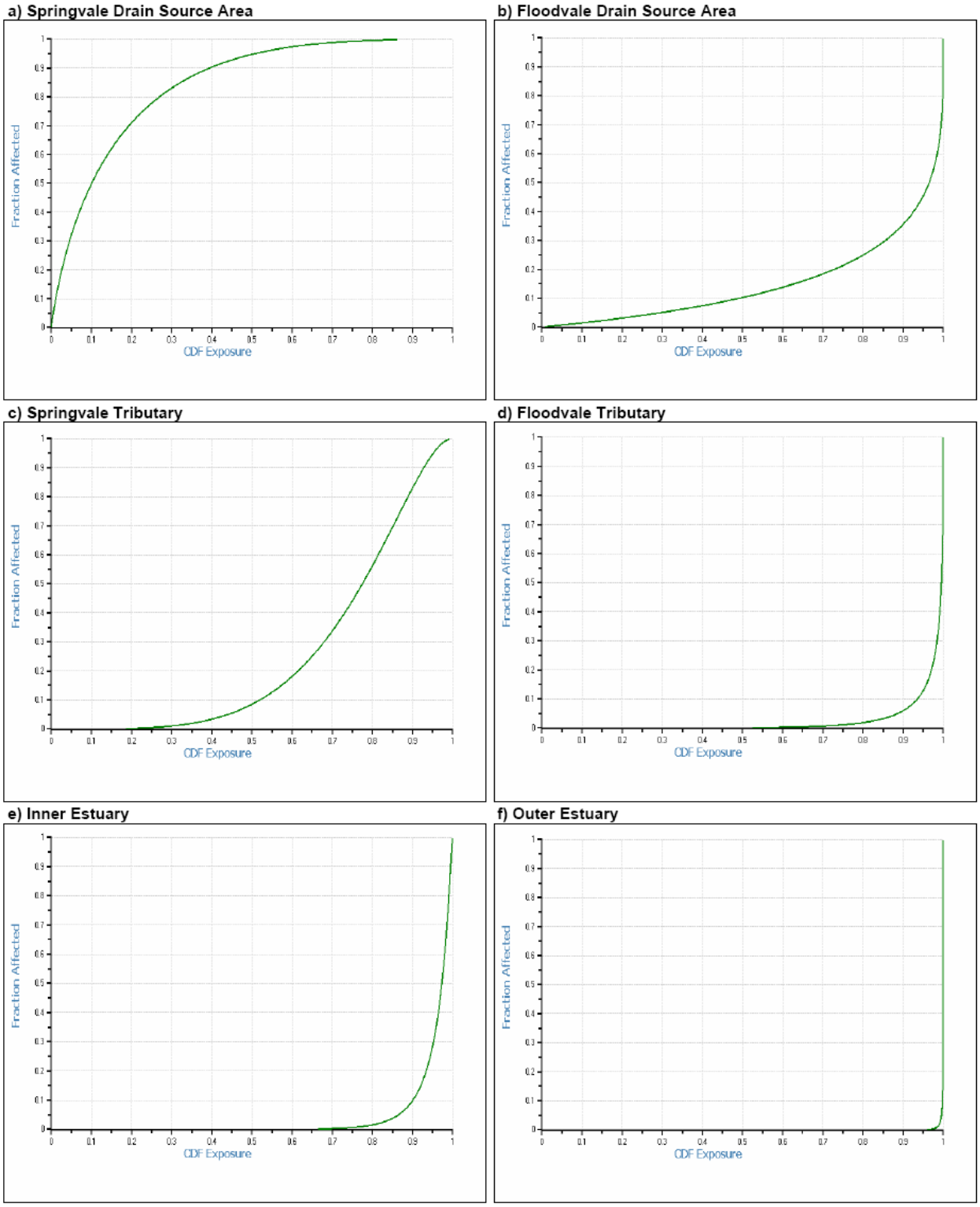


Figure 3a) to 3f). Joint Probability Curves (JPCs) for ecological risk in a) Springvale and b) Floodvale Source Areas and in Penrhyn Estuary in c) Springvale Tributary, d) Floodvale Tributary, e) Inner Estuary and f) Outer Estuary across both tides using the NOEC scenario.

curve shape associated with lower risk, with fewer species being adversely affected the majority of the time. In Springvale Drain >70% of species are affected at least 80% of the time, whereas in Floodvale Drain, <25% of species are affected at least 80% of the time. The ecological interpretation from the JPC curve is straightforward.

In Springvale Tributary, the risk was considerably lower than in the source area with the risk reduced from 84% to 25%. The JPC also shifted to a type where few species are adversely affected for the majority of the time, with <10% of species affected at least 50% of the time and >70% species being affected only <15% of the time (Figure 3c). In the Floodvale Tributary, the risk associated with discharge of groundwater decreased from 15% in the source area to only 2.3% in the estuary (Figure 3d). Both Springvale and Floodvale Tributaries discharge to the Inner Estuary. The increased influence of the discharge of water from Springvale Tributary over that of Floodvale Tributary is evident by the risk identified in the Inner Estuary ($\delta = 4$) being between the risk value identified for each of the tributaries. The JPC for the Inner Estuary reflected low risk, indicating that at least 90% of the time, less than 10% of species would be adversely affected by the contamination. The JPC for the Outer Estuary indicated almost no risk ($\delta = 0.1$) with >95% of species being protected >95% of the time.

The shape of the curve is informative in assessing type of exposure. In Springvale Drain, if it were an ecosystem for assessment, the type of community that might be expected to be present, given the shape of the JPC, would be a community dominated by low diversity, highly tolerant organisms. Given the constant exposure to the contaminants, this community would be expected to be variable. However, in the Springvale Tributary (Figure 3c), where there was a frequent exposure to moderately elevated concentrations of contaminants and occasional exposure to high concentrations with a high proportion of species being affected, it would be expected that the community would be characterised by moderately sensitive species. However, the community would also be moderately variable, given occasional elevated concentrations of contaminants. In the Outer Estuary, the JPC indicates that more than 95% of the time, all but the most sensitive species would be

protected (Figure 3f). This ecosystem would be characterised by a highly diverse and stable community. Based on guidance regarding the evaluation of the shape of the JPC curves (ECOFRAM 1999), the curve for Springvale Tributary posed an unacceptable risk profile, whilst the Inner Estuary, Floodvale Tributary and Outer Estuary all represented acceptable risk profiles. The benefit of the JPC methodology is that it can be used to derive ecological hypotheses for testing and provide a more targeted ecological assessment. Limited ecological sampling has been undertaken in the estuary, however, the limited sampling (from the Outer and Inner Estuary) indicated that the dominant taxa in the inner estuary in subtidal samples were polychaetes (Nereididae) and round worms (Nematoda), whereas in the outer estuary in intertidal samples were polychaetes (Nereididae) and amphipods (Exoedicerotidae) (TheEcologyLab 2003). Dominance of polychaetes and roundworms in the Inner Estuary may indicate the dominance of tolerant taxa.

Differences Between Toxicity Assessment Scenarios

Risk values for the EC50 scenario followed the same trends as the NOEC scenario; however, the magnitudes of risk values were typically smaller. The average risk (across the three exposure scenarios and the four locations) for the NOEC Scenario was 8%% (n = 12), whereas for the EC50 scenario, it was 4% (n = 12). The spatial distribution of risk was similar to the NOEC scenario with the greatest and lowest risk being identified in Springvale Drain and the Outer Estuary.

Differing magnitudes in the trends in risk were identified in each of the source areas. Only a small decrease in the risk of adverse effects was identified in Springvale Drain, decreasing from 84% to 55% when the EC50 scenario was compared to the NOEC scenario, whereas in the Floodvale Drain, there was a significant decrease in risk identified from 16% (NOEC) to only 4% (EC50). This difference in risk reflects the greater concentrations of VCHs in the Springvale Drain than in the Floodvale Drain. This assessment indicates however, that whilst there is a significant likelihood of strong adverse effects in Springvale Drain, there is very low likelihood of strong adverse effects in Floodvale Drain.

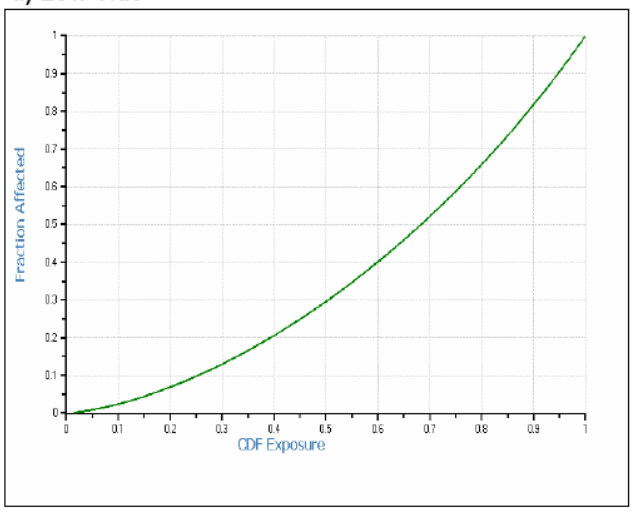
In the Springvale Tributary, the risk of possible adverse effects (NOEC Scenario) was on average 26% (using the three exposure scenarios; n = 3). When this risk was assessed for likely adverse effects (EC50 scenario), the risk was approximately halved (14%). In the other three locations within the estuary, the low risk of possible adverse effects (2%; n = 9, NOEC scenario) equated to a risk of likely adverse effects of <1% (EC50 scenario). This indicates that although there is a risk of possible adverse effects occurring, the risk of strong adverse effects is lower.

DIFFERENCES BETWEEN EXPOSURE ASSESSMENT SCENARIOS

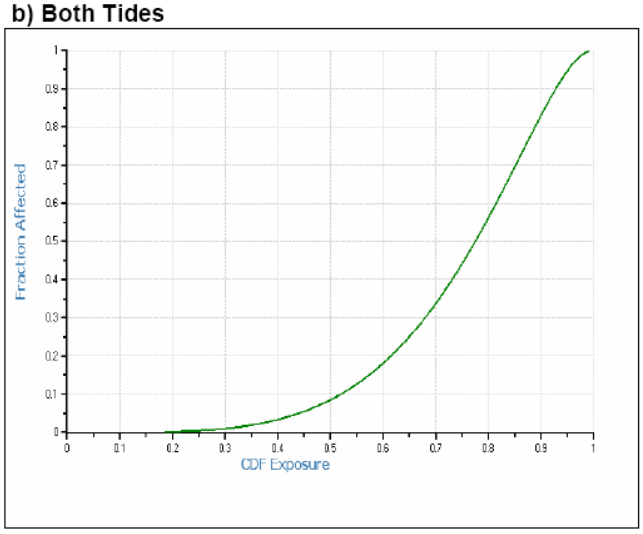
The greatest mean ecological risk for the NOEC scenario was reported at low tide (mean δ = 12, n=12), followed by both tides (mean δ = 8, n=12), whilst the least risk was reported at high tide (mean δ = 4, n=12). This interpretation of risk is consistent with the results from the hazard assessment, where concentrations of VCHs and risk are highest at low tide and lowest at high tide (Hunt *et al.* 2007). These results reflect the overall pattern reported in the screening level hazard assessment where risk at low tide > both tides > high tide (Hunt *et al. 2007*).

The total risk for the Springvale Tributary decreased from 36% at low tide (Figure 4a), to 25% across both tides (Figure 4b) to a minimum of 15% at high tide (Figure 4b). The shape of the JPCs is similar (albeit reflecting the greater area under the curve with the increased risk), however, the increased risk is reflected in the difference in the number of species affected in the exposure scenarios. In the high tide scenario, 50% of the time <5% of species may be affected, in the both tides scenario for 50% of the time approximately 10% of species may be affected. However, in the highest risk scenario, when only low tide data are assessed, approximately 50% of the time up to 30% of species may be affected. The increased risk results in a greater proportion of species being adversely affected through the tidal cycle. A small percentage of the time (~10%), between 60% and 80% of species would be affected when assessed in each of the three exposure scenarios.

a) Low Tide



b) Both Tides



c) High Tide

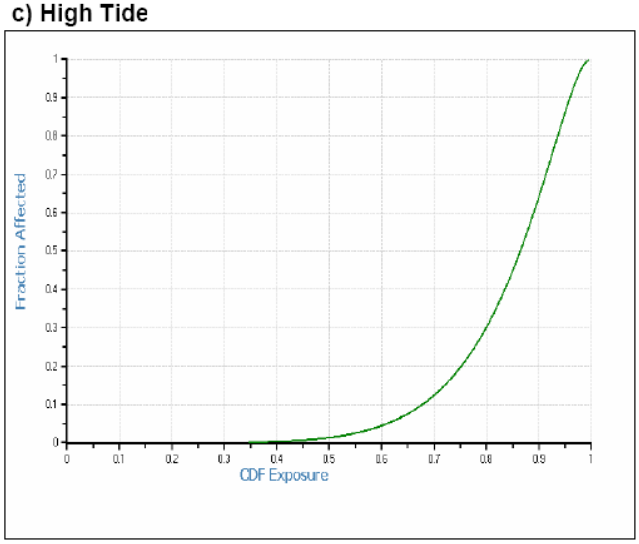


Figure 4a) to c). Comparison of JPCs across the tidal cycle in a) low, b) both and c) high tides in Springvale Tributary using the NOEC scenario.

The JPC for both tides is considered to represent the most ecologically relevant measure of ecological risk in the estuary, whereas the JPC for low tide only is expected to be an overestimate of ecological risk and the JPC for the high tide exposure only is likely to underestimate ecological risk. Organisms that are sessile within the estuary would be exposed to the full tidal cycle and therefore, their survival would be dependent on surviving the exposure to high concentrations at low tide. Given the difference in risk and the difference in fraction of species affected between the high and low tide scenarios, the survival of organisms and the diversity of the ecological community would be dependent on uptake and depuration rates of the VCHs. If organisms accumulate VCHs during low tide and are able to survive to depurate VCHs during high tide, with no decrease in survival, then a diverse community, including sensitive species would be present. However, if the organisms cannot depurate VCHs without a loss in survival, then a less diverse community, without sensitive species will be present. With that in mind, the site-specific SSD in the current study was derived for chronic exposure to VCHs (a conservative assessment), which is represented by the inclusion of both high and low tide data and uptake and depuration kinetics.

Comparison Of The Probabilistic ERA With The Screening Level Hazard Assessment

The current probabilistic ERA provided a higher tier risk assessment, using site-specific toxicity measures, of VCH contamination in Penrhyn Estuary. It is therefore, relevant to review the congruence in the assessments of risk between the current Probabilistic ERA and the screening level hazard assessment.

The risk assessment identified an unacceptable risk (>5%) in each of the source areas (i.e. Springvale Drain and Floodvale Drain), where an unacceptable hazard (HQ>1) had previously been identified. In Springvale Tributary, the hazard assessment identified unacceptable hazard (HQ>1) at all three tidal scenarios: low and high tides and both tides. In the current risk assessment, the assessment of risk was the same, with an unacceptable risk identified for all three tidal scenarios. In Floodvale Tributary, the hazard assessment identified unacceptable hazard (HQ>1) at low tide and across both tides, however, the

current risk assessment did not identify an unacceptable risk (>5%) in any of the three tidal scenarios. In the Inner Estuary, the hazard assessment identified unacceptable hazard (HQ>1) at low tide and across both tides, however, the current risk assessment only identified an unacceptable risk (>5%) at low tide. In the outer estuary, the risk assessment identified acceptable risk (<5%) where the hazard assessment had also identified an acceptable hazard (HQ<1).

The derivation of site-specific toxicity data and the incorporation of all available exposure and toxicity data and their inherent variability through the use of probability distributions for each factor has enabled a more accurate assessment of risk, indicating that, in the Floodvale Tributary and the Inner Estuary, where the previous assessment had identified a potential hazard, the more accurate assessment of risk indicated that this risk was acceptably low. In addition, the JPC provided a continuum of the number of species affected for a given proportion of the time, rather than 95% of species no longer being protected, as was provided in the hazard assessment. When determining ecological adversity, risk assessment should evaluate the nature and the intensity of effects, the spatial and temporal scale and the potential for recovery (USEPA 1998). The probabilistic approach undertaken in the current risk assessment provides this information on the type of exposure provided information on the likely types of organisms that may be present.

Additional Potential Site-Specific Applications Of The Risk Assessment Methodology

There are several advantages in the application of this probabilistic method to site-specific risk assessment over other methodologies. The current model can be used to remove confounding effects to assess the risk posed by VCHs alone, e.g. it can be used predictively, and as the basis of developing ecological hypotheses. Other benefits of the model are to undertake risk ranking for priority areas or assessing temporal changes in risk and it greatly assists in communication of risk to risk managers and stakeholders.

The JPC technique can be useful in removing some potential confounding associated with assessment of contamination. Penrhyn Estuary is hydraulically complex with the substratum composed of patchy areas of silt and sand, with variable organic carbon content, salinity and elevation. Each of these factors is known to affect the diversity and abundance of benthic communities, making it difficult to determine significant differences in ecological communities arising from VCH contamination. In addition, ecological interpretation may be difficult if no data are available for the pre-impact condition or if suitable reference areas cannot be identified. Alternatively, the assessment may be undertaken in an ecosystem where a number of confounding factors exist, including multiple contamination sources, where it is important to discriminate between the effects attributable to various sources, e.g. natural resource damage assessments (NRDAs) commonly undertaken in the United States. The methodology presented here provides a useful toxicologically-based measure of risk to resident communities without the potential confounding of other factors.

The risk methodology presented here also allows for assessment of risk *a priori*. The source of risk in this study is groundwater contaminated with VCHs migrating toward Botany Bay. The ecotoxicological model presented here could be coupled with a groundwater flow model, to predict ecological risk, given a number of scenarios regarding future migration of contaminated groundwater. This could include various contamination scenarios and timeframes for increases in ecological risk and requirements for remedial action. Similarly, the methodology could be used to quantify changes in risk associated with various remediation scenarios or management actions. The current methodology, which quantifies risk and the type of exposure of receptors, also improves communication of risk to managers and stakeholders through the provision of numbers for calculated risk and graphical output for the slope.

This is an improvement over simpler quantitative risk methods which cannot typically distinguish a frequent likelihood of a low consequence outcome and a rare likelihood of a catastrophic consequence (Verdonck *et al.* 2003). The current methodology provides

valuable information on the type of exposure, whether it is short exposure to high concentrations, with a diverse but variable community, or a prolonged exposure to low concentrations, resulting in a stable community, dominated by tolerant organisms. The slope of the risk curve, therefore, can assist in development of ecological hypotheses to evaluate the ecological condition.

Studies have recently been undertaken that derived field based SSDs for contaminated sediments (Kwok *et al.* 2008). In a sediment ERA, these SSDs could be incorporated into the current method and assessed spatially in which the quantified risk could be used to rank areas of priority for remedial action and reduction of risk. Alternatively, the current methodology could be used to assess temporal changes in risk to determine success of remedial actions and monitoring reduction of risk through time.

UNCERTAINTY

The purpose of including the discussion of uncertainty in the risk assessment is to inform risk managers and decision makers that uncertainty exists with the information presented. Although the treatment of uncertainty presented here only identifies sources of uncertainty and does not convey the potential extent or impact of the uncertainty associated with the risk assessment, it is nonetheless important to be explicit with all the sources of uncertainty to ensure that the risk assessment presented is completely transparent (Calow 1998).

Concentrations of VCHs within the waters of the estuary were highly variable; however, the toxicity assessment derived an SSD based on exposure to constant, concentrations of VCHs. Given the high water solubility, it is expected that VCHs would be taken up and depurated relatively quickly, however, this has not been quantified. It is considered that the derivation of the SSD at a constant concentration represents a worst case scenario as, unlike the estuary, the concentrations are not variable, and may not accurately reflect the variability in uptake and depuration rates.

Use of SSDs for the toxicity assessment, whilst widely accepted, carries intrinsic uncertainties including: extrapolation from a small number of species to all species; sensitivity of all species being log-normally distributed; and the presumption that protection of a proportion of the species present will be sufficient for protection of the ecosystem structure and function (Forbes and Forbes 1993). Toxicity testing as a means of assessing in-situ ecological risk will always be associated with uncertainty where toxicity tests are extrapolated to the real world. Solomon *et al.* (2000) reported that toxicity testing tends to overestimate risks when assessed in the environment, however, assessment of WET in North America indicated that where effluent (or in this case groundwater) was a large component of stream flow, as is the case in Springvale Tributary, WET testing indicated good predictability of toxicity and ecosystem effects (Waller *et al.* 1996).

During the derivation of the JPC, environmental data are fitted to a log normal distribution, however, 25% of the exposure distributions failed the Anderson-Darling test for normality ($P<0.05$). The implications of not meeting the assumption of log-normality are unknown. This could only be remedied with a methodology that did not involve assumption of a distribution type for exposure data.

Uncertainty exists with regard to what is an 'acceptable' level of risk. This study has quantified risk and compared risks for different areas of the estuary, however, the magnitude of the quantified ecological risk (δ) (i.e. what percentage risk constitutes a significant risk) and the ecological relevance of this, have not been validated in the field. Calibration of risk values (δ) with effects in ecosystems is required. It is noted, however, that 'acceptability' of levels of risk are the domain of risk managers and policy makers and not just science. Most of the potential sources of uncertainty identified in this study were equally applicable to the hazard quotient method, so, even with the uncertainty identified here, the current method represents a considerable improvement over the hazard quotient method.

CONCLUSIONS

The present study demonstrated the use of the JPC technique for site-specific ERA to characterise ecological risk of contamination in an estuary. This ERA demonstrated a quantitative, probabilistic ERA using probability distributions for exposure and toxicity assessments and with the extent of overlap of these distributions used to quantify ecological risk. The site-specific nature of the toxicity and exposure distributions greatly increase the relevance of the risk assessment.

Risk was greatest at low tide followed by a combination of both tides, with the lowest risk being reported at high tide. The two toxicity scenarios also allowed investigation of different levels of risk, i.e. for protection of organisms and possible ecological effects (NOEC) or assessment of significant adverse ecological effects (EC50).

The probabilistic methodology undertaken in this study represents a considerable improvement over the HQ approach including: quantification of the risk; information on the type of exposure for organisms in the receiving environment; and incorporation of the variability inherent in both toxicity and exposure datasets into the assessment of risk. This methodology can be used to develop testable ecological hypotheses on the resident ecological communities. Further work should be undertaken to validate the ecological significance of the quantified risk.

PAPER 5

USING A CRITICAL BODY RESIDUE APPROACH TO EVALUATE SPECIES SENSITIVITY AND THE ADDITIVE TOXICITY OF VOLATILE CHLORINATED HYDROCARBONS IN GROUNDWATER

ABSTRACT

Direct toxicity assessment (DTA) of volatile chlorinated hydrocarbon (VCH) contaminated groundwater and derivation of a site-specific guideline was undertaken using five indigenous marine species. VCHs have a non-polar narcotic mechanism of action. Such chemicals have consistently been observed to exert toxicity at a critical internal concentration or critical body residue (CBR) of approximately ~2.5 mmol/kg. Test organisms included: a micro-alga (*Nitzschia closterium*); an amphipod (*Allorchestes compressa*); a polychaete worm (*Diopatra dentata*); and sea urchin (*Heliocidaris tuberculata*) and oyster larvae (*Saccostrea commercialis*).

To evaluate the sensitivity of the test organisms, internal molar concentrations were calculated from toxicity testing of seawater spiked individually with 1,2-dichloroethane and chloroform. Internal lethal concentrations to 50% of organisms (ILC50) values varied from 0.007 to 5.50 mmol/kg. The mean ILC50s for 1,2-dichloroethane and chloroform were 2.32 mmol/kg and 2.84 mmol/kg respectively, which are close to the literature-based critical body residue (CBR) of ~2.5 mmol/kg. The lowest ILC50s occurred for the sub-chronic and chronic tests and endpoints.

Individual ILC50s for VCHs in contaminated groundwater varied from 0.19 to 2.11 mmol/kg, generally within the range expected for narcotic contaminants. The mean ILC50 of VCHs in contaminated groundwater was 0.88 mmol/kg (n=5) which was not significantly different ($P<0.05$) from mean ILC50s for spiked samples (n=5) of 2.32 mmol/kg and 2.84 mmol/kg.

It was concluded that, when assessed using the ILC50 and CBR approach, relative sensitivities of test organisms to VCHs were comparable with other test organisms and the additive internal concentrations of VCHs in groundwater was considered sufficient to account for the toxicity observed in the DTA.

INTRODUCTION

Groundwater contamination by volatile chlorinated hydrocarbons (VCHs) and industrial solvents is common internationally (USEPA 1990; Pohl *et al.* 2003; Zolezzi *et al.* 2005). Hunt *et al*. (2007) found that groundwater contamination with a complex mixture of VCHs that was entering Penrhyn Estuary, an embayment of Botany Bay, Sydney, Australia posed an unacceptable hazard. Direct toxicity assessment (DTA) of the groundwater and derivation of site-specific guidelines were reported in Hunt *et al*. (2009b) and a probabilistic risk assessment further characterised the ecological risk in the estuary (Hunt *et al. in press a*).

As many as 60% to 70% of all industrial organic chemicals are thought to exhibit a narcotic mode of toxic action (Veith *et al.* 1983; Bradbury and Lipnick 1990). Narcosis is the simplest and most common toxicity mechanism (Schultz 1989) and is described as the reversible, non-specific disturbance of membrane integrity and functioning as a result of partitioning of pollutants into membranes (van Wezel *et al.* 1995). Volatile chlorinated hydrocarbons (VCHs), such as those identified in the Penrhyn Estuary (Carey *et al.* 1998; Ren 2002), have a narcotic mode of action. The toxicity of such chemicals is dependent upon their hydrophobicity (Escher and Hermens 2002). Under the narcotic pathway, chemicals are essentially equipotent, based on internal molar concentrations at the target site, i.e. the membrane lipid.

The internal concentration of a contaminant in an organism, i.e. the concentration of the contaminant at the site of the receptor (i.e. cellular membranes), is considered to better reflect toxicity than external concentrations e.g. McCarty and Mackay (1993); and therefore, internal concentrations provide a better basis for assessing potential toxicity than external concentrations (Escher and Hermens 2002). Use of internal concentrations also removes potential confounding associated with the bioavailability, accumulation kinetics, and biotransformation of chemicals (McCarty and Mackay 1993b; van Wezel and Opperhuizen 1995; Lotufo 1998). The concentration at the site of action is related to the exposure concentration and for water only exposures, where there is only one route of

accumulation and behavioural modifications affecting uptake are minimal, the exposure concentration is a good surrogate (Lotufo 1998). There are currently no techniques available for measuring internal residues for VCHs in small organisms such as amphipods, unicellular algae and sea urchin and oyster larvae. Assessment of toxicity and concentrations at the site of action, therefore, relies on calculation of internal residues based on exposure concentrations in water and bioconcentration factors (BCFs).

The CBR was predicted to be ~2.5 mmol/kg wet weight (McCarty 1986). Experiments have found the CBR is consistent amongst species for acute toxicity, with measurements ranging from 1 to 10 mmol/kg wet weight for vertebrate and invertebrate species (van Hoogen and Opperhuizen 1988; Landrum *et al.* 1991; Sijm *et al.* 1993; van Wezel *et al.* 1995; van Wezel *et al.* 1996; van Wezel and Jonker 1998). The critical body burden model has been extended to the target lipid model (TLM), effectively normalizing the CBR to the lipid content of the organism (Di Toro *et al.* 2000; McGrath *et al.* 2004). Ranges of lipid normalised values of toxicity for algae are equivalent to CBRs of between 0.73 and 14.3 mmol/kg wet weight (McGrath *et al.* 2004). Whilst numerous studies identified the consistent range of between 1 mmol/kg and 10 mmol/kg for acute lethality, few studies have examined more subtle endpoints. Lotufo (1998), however, using the CBR model, identified lethality in the range of 1.2 mmol/kg to 2.7 mmol/kg, and sub-lethal responses including: a reduction in offspring at residues of 0.5 mmol/kg; and a reduction in grazing rate at concentrations as low as 0.2 mmol/kg.

According to the mixture toxicity scheme of Plackett and Hewlett (1952) there are four types of joint action between components of mixtures. Theoretically when chemicals with the same mechanism of action are combined they should conform to concentration addition (CA) (i.e. the combined effect of the components is equal to the sum of the concentrations of each chemical expressed as a fraction of its own individual toxicity). This has been confirmed experimentally using laboratory-based (Deneer *et al*., 1988; Hermens *et al*., 1984; 1985; Broderius and Kahl, 1985) and field-based (Dyer *et al.* 2000) aqueous toxicity data (Warne and Hawker 1995; Di Toro and McGrath 2000; Di Toro *et*

al. 2000; Broderius *et al.* 2005). Warne and Hawker (1995) found that as the number of components in equitoxic mixtures (mixtures where each component is present at the same fraction of their own individual toxicity) increases, the deviation from toxic additivity decreases. Van Wezel *et al*. (1996) demonstrated that the laboratory-based toxicity of mixtures of non-polar narcotics when expressed in terms of internal critical body residue concentrations conformed to CA. This was subsequently found to also apply to field-based mixtures of non-polar narcotic compounds (van Loon *et al.* 1997; van Wezel and Jonker 1998).

Hazard and risk assessments are almost exclusively based on external effect concentrations (i.e. in water). When expressed in these terms uptake and toxicity data depend on the species, tests, exposure and bioavailability of the contaminant (Escher and Hermens 2002). Consideration of internal effect concentrations may be more appropriate for risk assessment than consideration of external concentrations (van Wezel and Jonker 1998; Connell *et al.* 1999).

DTA was undertaken on the groundwater containing a complex mixture of VCHs using a battery of five indigenous test organisms and derive site-specific water quality guidelines (Hunt *et al.* 2009b), however, the relative sensitivity of these test organisms is unknown. The ANZECC & ARMCANZ (2000) water quality guidelines (WQGs) recommend that following DTA, a toxicity identification evaluation (TIE) should be undertaken to identify the likely cause of the observed toxicity. TIE, however, requires additional laboratory expertise and cost, and the test may not be able to identify all components of a complex mixture of contaminants contributing to toxicity (van Loon *et al.* 1997), particularly if the test medium potentially contains a number of unknown organic contaminants.

The objectives of the current study were to: determine if the critical body residues of chloroform and 1,2-dichloroethane in five indigenous Australian marine test organisms were consistent with published values (i.e. between 1 and 10 mmol/kg) for non-polar

narcotics; and determine using the critical body residue approach whether VCHs account for the majority of the toxicity observed in the groundwater DTA in Hunt *et al*. (2009b).

METHODOLOGY

TOXICITY TESTING

Toxicity testing was undertaken on the five test organisms using seawater samples spiked with known concentrations of 1,2-dichloroethane and chloroform to determine the relative sensitivity of test organisms used in the DTA and quantitative ecological risk assessment (Hunt *et al.* 2009b*; in press a*). To prevent the loss of volatile contaminants from test containers, testing was undertaken in sealed containers using the methodology presented in Hunt *et al*. (2009a).

Chloroform and 1,2-dichloroethane were purchased from Lab Scan Analytical Services (AR Grade, 99.8% purity). For each chemical, a stock solution was made and serially diluted with seawater six times, by a factor of three. Exposure concentrations during toxicity testing were measured. Exposure concentrations varied from ~0.05 mg/L to ~800 mg/L and from ~0.003 mg/L to ~200 mg/L for 1,2-dichloroethane and chloroform, respectively.

To evaluate the exposure of test organisms, samples were collected from an additional test vessel containing test solutions, but no test organisms, Samples were collected in 40 mL glass vials with airtight Teflon™-lined lids with zero headspace, immediately preserved with hydrochloric acid and stored at <4°C. Samples were extracted using purge and trap methodology (USEPA 5030B) and analysed by Gas Chromatography and Mass Spectrometry (GC/MS) utilising a modification of USEPA Method 8260B for volatile organic compounds (USEPA 1996c). The limit of reporting was 1 µg/L for all analytes with the exception of vinyl chloride (10 µg/L). Quality control evaluations were undertaken on each sample batch. No analytes were detected in method blanks and recoveries for laboratory control samples and matrix spikes were between 80% and 120%, and within accepted criteria. Differences between primary and duplicate samples were generally <25% and typical of variability between duplicate samples for VCH analysis at this laboratory (URS 2004a).

A detailed description of the toxicity testing undertaken in the DTA is presented in Hunt *et al*. (2009b), however, a brief summary of the methodology is provided. The DTA involved the collection of a groundwater sample from an industrial facility that was known to be contaminated with at least 14 VCHs including carbon tetrachloride, vinyl chloride, 1,2-dichloroethane and chloroform (Table 1). The 50% dilution of the groundwater mixture contained approximately 45.5 mg/L total VCHs and was adjusted to marine conditions (~35 ppt).

Toxicity testing and endpoints included: a 72 hour population growth inhibition test (cell yield) on the benthic alga (*Nitzschia closterium*); 72 hour sea urchin (*Heliocidaris tuberculata*) and oyster (*Saccostrea commercialis*) normal larval development tests; and 96 hour survival tests using a polychaete worm (*Diopatra dentata*) and an amphipod (*Allorchestes compressa*). The algal growth test is a chronic test. The oyster and sea-urchin larval development tests are sub-chronic tests and the amphipod and polychaete tests are acute tests. Toxicity tests for small organisms, i.e. algal growth and sea urchin and oyster larval development tests, were undertaken in sealed 44 mL vials with Teflon™-lined lids, whereas toxicity tests with medium sized organisms, i.e. polychaete and amphipod tests, were undertaken in sealed 1 L jars with Teflon™-lined lids. Vials contained no headspace however, jars contained between 40% and 50% headspace. For each test, temperature, pH, salinity and dissolved oxygen content of a sample from each treatment were measured at the start; immediately prior to renewal of test water; and at the conclusion of the test.

Calculation of Critical Body Residues

All measures of toxicity were initially expressed in terms of aqueous concentrations. Concentrations of contaminants affecting 50% of test organisms or causing a 50% effect (LC50 and EC50 values) were determined by the trimmed Spearman-Karber Method using TOXCALC™ V5 (Tidepool™ Scientific Software). No observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values were determined by performing Dunnett's or Steel's Many-One Rank tests, depending on the distribution of the data using TOXCALC™ V5 (Tidepool™ Scientific Software).

Bioconcentration factors (BCFs) were calculated by:

$$\mathbf{BCF = C_{organism} / C_{water}} \quad \mathbf{(1)}$$

Where $\mathbf{C_{organism}}$ represents the concentration of contaminant in the organisms and $\mathbf{C_{water}}$ represents the concentration of contaminant in the surrounding water.

Internal critical body residues (i.e. IEC50 and ILC50 in mmol/kg) were calculated by:

$$\mathbf{ILC50\ (CBR) = LC50 \times BCF} \quad \mathbf{(2)}$$

Bioconcentration factors were obtained for each contaminant present in the groundwater mixture from literature sources and the hazardous substances database (HSDB) (available at *www.toxnet.nlm.nih.gov*) (Table 1).

The most common method for assessing addition of toxicity is the toxic unit (TU) approach represented by:

$$\mathbf{\Sigma\ TU = C_i / EC}x_i \quad \mathbf{(3)}$$

where, C_i is the concentration of component *i* and $EC x_i$ is the concentration of component *i* that elicits effect *x* (Altenburger *et al.* 2000). When ΣTU, is equal to one, concentration addition occurs. Where ΣTU is less than one, toxicity is more than additive and where ΣTU is greater than one, toxicity is less than additive (Nirmalakhandan *et al.* 1997; Broderius *et al.* 2005). The TU approach is, however, reliant on the availability of individual toxicity metrics, i.e. EC50 values, being available for all species tested for each of the components of a mixture, which is onerous where toxicity testing has been undertaken on indigenous species or contaminants for which scant toxicological information is available. In the current study, insufficient data (i.e. EC50/LC50 values) are available for each of the components of the groundwater mixture, however, the individual internal molar residue of each component of the groundwater mixture can be added to evaluate the contribution of each of the components to the overall toxicity.

Table 1. Bioconcentration factors (BCFs) of volatile chlorinated hydrocarbons (VCHs) identified in groundwater.

Volatile Chlorinated Hydrocarbons	BCF
Vinyl chloride	6.00
1.1-Dichloroethene	2.5
1.1-Dichloroethane	5.00
cis-1.2-Dichloroethene	5.00
Carbon Tetrachloride	22.8
1.2-Dichloroethane	2.00
Trichloroethene	16.2
1.1.2-Trichloroethane	10.0
Tetrachloroethene	51.5
1.1.2.2-Tetrachloroethane	8.63
Chloroform	6.63

RESULTS AND DISCUSSION

SENSITIVITY OF THE TEST ORGANISMS

In the toxicity testing of seawater spiked with 1,2-dichloroethane, EC50/LC50 values varied from a minimum of 17.5 mg/L (algal growth test) to a maximum of 244 mg/L (amphipod survival test) (Table 2), which equated to ILC50s of between 0.36 mmol/kg (algal growth test) and 5.00 mmol/kg (amphipod survival test) (Table 2). For toxicity testing on seawater spiked with chloroform, EC50/LC50 values varied from a minimum of 0.12 mg/L (sea urchin larval development test) to a maximum of 98.8 mg/L (oyster larval development test). Individual ILC50s varied between 0.007 mmol/kg (for the sea urchin larval development test) to 5.5 mmol/kg (for the oyster larval development test). Individual ILC50s were within the range of 1 and 10 mmol/kg identified by van Wezel and Jonker (1998) for 70% of toxicity tests. ILC50 values were outside the range of 1 and 10 mmol/kg for the algal test (for both 1,2-dichloroethane and chloroform) and the sea urchin larval development tests (for chloroform only). ILC50s for the sea urchin test (0.007 mmol/kg for chloroform) and the algal growth test (0.036 mmol/kg and 0.012 mmol/kg for 1,2-dichloroethane and chloroform, respectively), were nearly two orders of magnitude less than the values for the other test species. Meador (2006) noted that although predictions of lethality were associated with high tissue residues (i.e. ~2.5 mmol/kg), at lower residues, other effects, for example reduced growth, impaired reproduction or abnormal development may be observed. The lower ILC50 values of the sensitive endpoints for the algal (population growth) and sea urchin (larval development) tests are consistent with Lotufo's (1998) estimates of IEC50s of sub-lethal responses, for example, a reduction in offspring at residues of 0.5 mmol/kg and a reduction in grazing rate at concentrations as low as 0.2 mmol/kg.

The variability in the individual ILC50 values is influenced by a number of factors, including the toxicokinetics (i.e. the uptake, distribution, metabolism and excretion of VCHs). It would be expected that toxicity values would be spread over a smaller range if the concentrations were normalised to the lipid content of the organisms preferably differentiating between storage and membrane lipids, and if the toxicant concentration

Table 2. Effect concentrations (EC50/LC50), bioconcentration factors (BCF) and predicted internal lethal residues for 50% of test organisms (ILC50) exposed to a mixture of volatile chlorinated hydrocarbons in groundwater and seawater spiked individually with 1,2-dichloroethane and chloroform.

Test organisms		Groundwater				1,2-dichloroethane				Chloroform			
		External LC50		BCF	CBR	External LC50		BCF	CBR	External LC50		BCF	CBR
		mg/L	mmol/L	--	mmol/kg	mg/L	mmol/L	--	mmol/kg	mg/L	mmol/L	--	mmol/kg
Nitzschia closterium	Alga	4.10	0.041	--	0.19	17.5	0.18	2.00	0.36	0.21	0.00	6.625	0.012
Heliocidaris tuberculata	Sea Urchin	3.77	0.038	--	0.17	55.8	0.57	2.00	1.14	0.12	0.00	6.625	0.007
Saccostrea commercialis	Oyster	9.79	0.099	--	0.45	198	2.02	2.00	4.04	98.8	0.83	6.625	5.50
Diopatra dentata	Polychaete	32.08	0.325	--	1.49	52.6	0.54	2.00	1.07	80.7	0.68	6.625	4.49
Allorchestes compressa	Amphipod	45.50	0.460	--	2.11	245	2.50	2.00	5.00	74.8	0.63	6.625	4.17
Average					0.88				2.32				2.84

-- Values for BCFs in groundwater are provided in Table 1.

were measured in the target tissue (Escher *et al*., in press). Although the internal ILC50 should be consistent for a test organism over time, there is difference between the ILC50s for test organisms estimated using ambient concentrations, as in this study, as uptake will be dependent on the exposure concentration and transfer across lipid membranes (Escher *et al*., in press).

The mean ILC50 for 1,2-dichloroethane and chloroform were 2.32 mmol/kg and 2.84 mmol/kg (n = 5), close to the value of 2.5 mmol/kg predicted by McCarty (1986) and within the range of 1 and 10 mmol/kg identified by van Wezel and Jonker (1998) (Figure 1). It is not surprising that the mean ILC50s of each of 1,2-dichloroethane and chloroform were consistent with the predicted mean CBR of 2.5 mmol/kg, given that the majority of toxicity values were within the range identified by van Wezel and Jonker (1998). The test organisms are, therefore, considered to be appropriately sensitive to VCHs and suitable for use in the derivation of site-specific guidelines (Hunt 2009b). Relative sensitivity of individual tests, however, reflected the sensitivity of the endpoints selected.

EVALUATION OF THE ADDITIVITY OF THE TOXICITY OF VCHS IN GROUNDWATER

The ILC50 of the mixture of VCHs in groundwater was calculated to assess whether the VCHs accounted for the toxicity observed in the DTA (Hunt *et al.* 2009b). It was hypothesized that if ILC50 values for the groundwater sample containing a complex mixture of VCHs were not significantly different to those derived for the individual spike tests, (i.e. 1,2-dichloroethane and chloroform assessed individually), and if the ILC50s were within the range expected for narcotic contaminants, then the toxicity could be attributed to VCHs. If however, ILC50s derived for the groundwater (i.e. the mixture of VCHs), were significantly different to those derived for the individual spike tests of 1,2-dichloroethane and chloroform, and not within the range of ILC50 values for narcotic contaminants, then the observed toxicity of the groundwater would not be attributable to the complex mixture of VCHs.

EC50/LC50 values for the contaminated groundwater varied from a minimum of 3.77 mg/L total VCHs (sea urchin larval development test) to a maximum of >45.5 mg/L (amphipod survival test). The corresponding ILC50s varied from 0.17 mmol/kg (sea urchin larval development test) to a maximum of >2.11 mmol/kg (amphipod survival test). The EC50 and resulting ILC50 were identified as '>' for the amphipod survival test as the highest concentration tested (50% groundwater) did not elicit a toxic response.

Individual ILC50s for the two acute lethality toxicity tests (polychaete and amphipod survival) were 1.49 mol/kg and 2.11 mmol/kg respectively, within the expected range of between 1 mmol/kg and 10 mmol/kg for lethality. ILC50s for the sub-chronic and chronic tests for sensitive endpoints were 0.17 mmol/kg (sea urchin larval development test), 0.45 mmol/kg (oyster larval development test) and 0.19 mmol/kg (algal population growth test). These were similar to the values of 0.2 mmol/kg and 0.5 mmol/kg for grazing and reduced fecundity identified by Lotufo (1998). The individual ILC50 values for the groundwater were also similar to ILC50 values identified for individual contaminants (1,2-dichloroethane and chloroform) using spiked seawater for all test organisms, with the exception of the oyster larval development test, where the ILC50 for the groundwater (0.45 mmol/kg) was approximately one order of magnitude less than the ILC50 for both 1,2-dichloroethane (4.04 mmol/kg) and chloroform (5.50 mmol/kg). The ILC50 values for the individual tests for the mixture of VCHs in groundwater would be subject to the same sources of variability as the seawater spike tests, i.e. differences in lipid content and toxicokinetics.

The mean ILC50 for groundwater was 0.88 mmol/kg total VCH (n = 5), marginally less than the range of 1 and 10 mmol/kg for narcotic chemicals identified by Wezel and Jonker (1998) and marginally less than the mean ILC50 values of 2.32 mmol/kg and 2.84 mmol/kg for 1,2-dichloroethane (n=5) and chloroform (n=5), respectively (Figure 1). The mean ILC50 value for groundwater was, however, not statistically significant different from ILC50s for the two spike seawater tests when assessed using a one way analysis of variance ($P<0.05$). Approximately 90% of the total contribution to the ILC50 was

attributed to only four contaminants: 1,2-dichloroethane (40%); vinyl chloride (20%); trichloroethene (20%); and tetrachloroethene (10%).

Given that the ILC50 for the test species exposed to the mixture of VCHs in groundwater was not significantly different to the ILC50s identified for the test species in individual spike tests and as the range of individual ILC50s for the test species were within the ranges of ILC50s expected for narcotic contaminants, it is concluded that the toxicity of the sum of the individual narcotic contaminants in groundwater was sufficient to account for the toxicity observed in the DTA. Given the extensive contamination identified in groundwater at the industrial facility, it is possible that other VCHs or narcotic contaminants may be present in groundwater, possibly at concentrations less than their respective limits of reporting that could also contribute to the observed toxicity of the mixture. If it were possible to characterize these chemicals, it may slightly increase the predicted ILC50 of the mixture in groundwater. The advantage of DTA presented in Hunt *et al*. (2009b), however, is that the direct toxicological measurement allowed potential toxic effects exerted by unknown contaminants to be incorporated into the toxicity assessment.

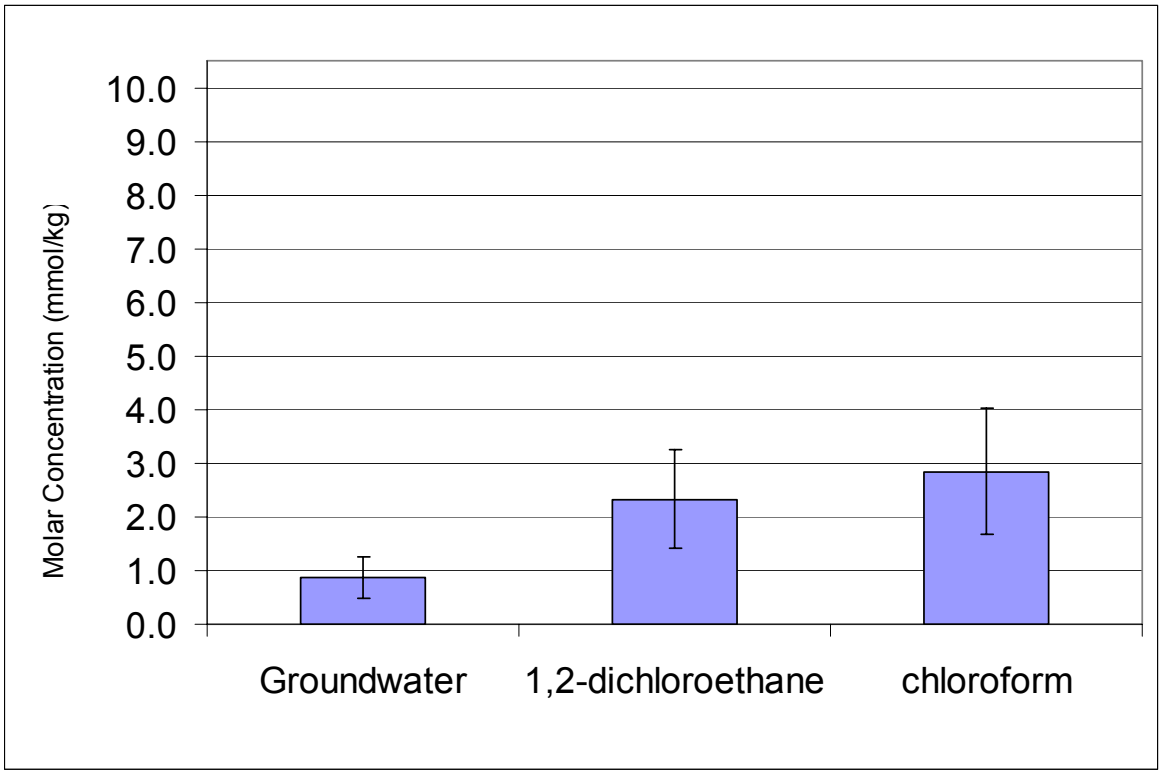


Figure 1. Mean predicted internal lethal residues to 50% of test organisms (ILC50) for volatile chlorinated hydrocarbons (VCHs) in groundwater and seawater samples spiked individually with 1,2-dichloroethane and chloroform. Error bars indicate ±S.E.

CONCLUSIONS

The present study used the critical body residue (CBR) concept to assess the relative sensitivity of five indigenous test species for which previously little toxicity testing data for VCHs was available. ILC50s were also used to assess the toxicity of a complex mixture of VCHs in groundwater.

ILC50s for individual tests varied, depending on the sensitivity of the test endpoint, however, the mean ILC50s indicated that sensitivity of the organisms was within the expected ranges and close to the predicted value of ~2.5 mmol/kg. Organisms were generally not considered to be overly sensitive or insensitive and were therefore, considered suitable for use in toxicity testing, i.e. the direct toxicity assessment (DTA).

When the ILC50 value predicted from additive components of the contaminated groundwater was assessed, it was not significantly different from the ILC50s from individual spike tests and was generally within the expected range for narcotic contaminants. The additive toxicity of VCHs in groundwater was, therefore, considered to account for the toxicity observed in the DTA in Hunt et al. (2009b).

Assessment of predicted CBRs based on exposure concentrations and bioconcentration factors provided a suitable, cost-effective method to evaluate the potential toxicity of a contaminant mixture, without the need to undertake additional toxicity testing or evaluation.

PAPER 6

DERIVATION OF WATER QUALITY GUIDELINES FOR 1,2-DICHLOROETHANE AND CHLOROFORM USING THE ANZECC AND ARMCANZ (2000) METHOD

ABSTRACT

Toxicity testing was undertaken to evaluate the existing *low reliability* trigger values (TVs) for 1,2-dichloroethane and chloroform provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Six indigenous Australian marine species were tested including: an alga (*Nitzschia closterium*); an urchin (*Heliocidaris tuberculata*); an oyster (*Saccostrea commercialis*); a fish (*Macquaria novemaculeata*); an amphipod (*Allorchestes compressa*); and a polychaete (*Diopatra dentata*). No observed effect concentrations (NOECs) for 1,2-dichloroethane varied from 580 to 159 000 µg/L and for chloroform, they varied from 4 to 55 200 µg/L. The objectives of the study were i) to evaluate if the existing TVs are protective of indigenous marine species and ii) to derive new TVs using the data generated in the toxicity testing.

To assess the first aim, NOECs derived in the present study were compared to the current TVs for chloroform (770 µg/L) and 1,2-dichloroethane (1900 µg/L). NOECs for the sea urchin larval development and algal population growth tests were less than the TVs, indicating that the TVs were not protective of these species.

Species sensitivity distributions (SSDs) and new TVs were derived using NOEC data generated from the current study, and the methodology in Australian and New Zealand water quality guidelines. New TVs that should protect 95% of species were derived for 1,2-dichloroethane (i.e. 165 µg/L) and for chloroform (i.e. 3 µg/L). These are between one and two orders of magnitude less than the existing *low reliability* TVs. Evaluation of ANZECC and ARMCANZ (2000) TVs for other volatile chlorinated hydrocarbons is required.

INTRODUCTION

Water quality guidelines, typically provide 'safe' concentrations for chemicals in the environment that should be protective of aquatic species, which in Australia, are contained within the Australian and New Zealand Guidelines for Fresh and Marine Water Quality (2000). These guidelines use two methods to calculate the 'safe' concentrations for toxicants which are termed 'trigger values' – if they are exceeded further action is triggered. The first, the Assessment Factor (AF) method relies on selecting the most sensitive species and dividing by an assessment factor (AF). This method is used where data for few test species are available. The second method the Burr Type III species sensitivity distribution (SSD) method (Shao 1990) derives a distribution of all species in an environment and predicts a concentration associated with a desired level of protection (i.e. percentage of species to be protected). The SSD method is more data intensive; however, it is increasingly being used in derivation of water quality guidelines and in conducting ecological risk assessments throughout the world (Posthuma *et al.* 2002; Wheeler *et al.* 2002; Schmitt-Jansen and Altenburger 2005). A framework for selection of the appropriate guideline derivation method is provided ANZECC and ARMCANZ (2000).

A variety of different parametric and non-parametric methods have been used to derive SSDs (van der Hoeven 2001; Posthuma *et al.* 2002; van Straalen 2002; Maltby *et al.* 2003). In Australia, however, the Burr Type III distribution, a flexible 3 parameter distribution was adopted for use (ANZECC and ARMCANZ 2000; Warne 2001). In addition to choice of distribution, debate has also focussed on the number of species required for derivation of an SSD. Newman *et al.* (2000) concluded that approximately 30 species were required to decrease the variability in the derived SSD; however, this is considerably greater than the number required by most international regulatory guidance, with at least 5 species required in the Netherlands, between 5 and 8 required by the OECD, at least 8 required in the USA, 5 species required in Australia and New Zealand (Warne 2001) and 10 for EU member countries (ECB, 2003). Wheeler *et al.* (2002) suggested 10 test species and Newman *et al.* (2000) recommended between 15 and 55, with 30 being the

optimal number to minimise variation in the derived SSD. Greater numbers would lead to smaller confidence intervals (Hose 2005).

One benefit of the SSD method is that a desired level of protection, for example, the PC95 which should theoretically protect of 95% of species in the ecosystem being examined, can be selected for a risk based approach to the assessment and management of water quality. In ANZECC & ARMCANZ (2000), TVs are provided to protect 99%, 95%, 90% and 80% of species (i.e. the PC99, PC95, PC90 and PC80 respectively) depending on the current ecosystem condition, with the PC95% being applied to 'slightly to moderately disturbed'(ANZECC and ARMCANZ 2000). Derivation of water quality guidelines using SSDs is reliant on the validity of the notion that ecosystem structure and function will be protected if *x*% of species are protected. Although this notion has been criticised, studies by Versteeg *et al.* (1999), van den Brink *et al.* (2002) and Hose and van den Brink (2004) suggest that selection of a hazard concentration protecting 95% of the single species sensitivity distribution appears to provide an appropriate level of protection when compared to multi-species or field studies.

In the ANZECC & ARMCANZ (2000) Guidelines, TVs for volatile chlorinated hydrocarbons (VCHs) were classed as *low reliability* as the SSDs were derived using chronic NOEC toxicity modelled from quantitative structure activity relationships (QSARs). Although various studies have assessed the sensitivity of Australian species compared to species from overseas (see Rose *et al.*, (1997) for a detailed account), only one study by Rose *et al.* (1998) examined organics and this focussed on the single species, *Ceriodaphnia cf dubia*. To date, no evaluation of TVs for VCHs has been undertaken for indigenous Australian species. A rigorous process for the assessment of data quality was undertaken prior to derivation of the TVs (Warne and Westbury 1999); however, in order to be protective to Australian species and account for the variability in the QSAR, a safety factor of 10 was applied to modelled data in derivation of the TVs (Warne 2001).

A screening level hazard assessment was undertaken of VCH contamination in an estuarine embayment in Sydney, Australia (Hunt *et al.* 2007). A key limitation of the

hazard assessment was identified as reliance on *low reliability* TVs for VCHs in the toxicity assessment (Hunt *et al.* 2007). Validation of these *low reliability* guidelines has been identified as a key priority for research (ANZECC and ARMCANZ 2000). Two of the VCHs identified as exceeding TVs in the hazard assessment presented in Hunt *et al.* (2007) were 1,2-dichloroethane and chloroform. Therefore, these contaminants were selected for toxicity testing to develop toxicity data for Australian marine species to evaluate the existing *low reliability* TVs. Chloroform and 1,2-dichloroethane are both volatile chlorinated alkanes with high Henry's Law Constants, low K_{ow} and K_{oc} values (Table 1). As such, they are water soluble, do not bioaccumulate and are readily taken up and depurated by organisms (Carey *et al.* 1998). Their mode of action is narcosis (non-specific or baseline toxicity), defined by Abernathy *et al.* (1988) as the general disruption of membrane associated metabolic activities. Narcosis is reversible and is used interchangeably with the term anaesthesia (Bradbury *et al.* 2003).

The objectives of the current study were: i) to evaluate if the current Australian and New Zealand (ANZECC and ARMCANZ 2000) *low reliability* TVs for 1,2-dichloroethane and chloroform are protective of indigenous species and to compare the sensitivity of indigenous marine species with the modelled QSAR species used in deriving the TVs; and ii) to derive new, higher reliability TVs for 1,2-dichloroethane and chloroform using experimental data to replace the existing *low reliability* TVs.

Table 1. Physicochemical Properties of 1,2-dichloroethane and chloroform

Property	**1,2-dichloroethane**	**chloroform**
Molecular Weight	98.96	119.38
Aspect	Clear liquid	Clear liquid
Melting Point	-35.5°C	-63.5°C
Boiling Point	83.5-84.1°C	60.5-61.2°C
Density	1.23-1.25 g/cm^3 20°C	1.476-1.478 g/cm^3 20°C
Vapour Pressure	8700 hPa (20°C)	211 hPa (20°C)
Log octanol-water Partition Coefficient (K_{ow})	1.45	2.0
Organic-carbon Partition Coefficient (K_{oc})	19-125	63.4-86.7
Water Solubility	8.5-9.0 g/L	8.0 g/L
Henry's Law Constant	110 Pa.m^3/mol	315 Pa.m^3/mol

METHODOLOGY

TEST WATER PREPARATION

Filtered seawater (FSW) was obtained from a clean source at Lurline Bay, Sydney, Australia and filtered to 0.45µm. Chloroform and 1,2-dichloroethane were purchased from Lab Scan Analytical Services (AR Grade, 99.8% purity). A stock solution of each chemical was made up by adding an appropriate amount of the chemical to 2 L volumetric flasks, which was then filled with FSW.

NUMBER AND SELECTION OF TEST SPECIES

Six indigenous organisms were selected for toxicity testing and evaluation, in accordance with the approach outlined in ANZECC and ARMCANZ (2000). The selected species are representative of temperate marine ecosystems of south-eastern Australia, are ecologically relevant and some have commercial or recreational value. *Saccostrea commercialis* (Sydney Rock Oyster) is farmed commercially in south-eastern Australia. Amphipods, including *Allorchestes compressa*, are the dominant macroscopic group on reef surfaces and constitute the dominant dietary component of small (0.1 to 100 g) inshore fishes (Edgar 1997). *Heliocidaris tuberculata* (sea urchin), *Macquaria novemaculeata* (Australian bass) and *Diopatra dentata* (polychaete worm) are all commonly found in temperate waters of south-eastern Australia. The test animals are also from a variety of trophic levels i.e. primary producers (*N. closterium*), grazers (*H. tuberculata* and *A. compressa*), a filter feeder (*S. commercialis*) and a detritivore (*D. dentata*). As narcosis is the mode of action for VCHs, all test species should be sensitive to the mode of action of both contaminants. Of the test species identified above, *D.dentata* had not previously been used in toxicity testing in the published literature and *M.novemaculeata* had only been used in two published studies (Cohen *et al.* 2000; 2003).

TOXICITY TESTING

Chloroform and 1,2-dichloroethane would be quickly lost from test solutions if the test vessels were left open to the atmosphere. Toxicity tests were therefore, undertaken in sealed vessels to prevent loss of VCHs and to maintain constant exposure concentrations in

accordance with the methodology presented in Hunt *et al.* (2009a). A summary of the methodology detailed in Hunt *et al.* (2009a), is provided below, with the test methodology for each of the test species.

Toxicity testing of small organisms (i.e. urchin and oyster larvae and the alga) was undertaken in 44 mL glass vials with Teflon™ lined lids and zero headspace. Each toxicity test consisted of seven threefold dilutions, each conducted in quadruplicate with concentrations of 1,2-dichloroethane varying from 1.0 to 1000 mg/L and chloroform varying from 0.3 mg/L to 3000 mg/L. Test solutions were not renewed for the duration of testing (72 h). Toxicity tests with larger organisms (i.e. amphipods, larval fish and juvenile polychaetes) were undertaken in 1 L jars with 500 mL of test solution and sealed with Teflon™ lined lids. Each toxicity test consisted of a control and four threefold dilutions, each conducted in triplicate, with concentrations of 1,2-dichloroethane varying from 30 to 1000 mg/L and chloroform varying from 10 to 300 mg/L. Test solutions in jars were renewed at the mid-point of testing (i.e. 48 h). Toxicity test conditions for test organisms are summarised in Table 2. A filtered seawater (FSW) control was undertaken for each toxicity test. Reference toxicants were undertaken for all test organisms with the exception of: the larval fish, for which the ethics approval for the project seeks to limit the number of organisms used and does not support reference toxicant testing; and the polychaete, which has not previously been used before and for which no reference toxicant information is available. Temperature, pH, salinity and dissolved oxygen content of a representative sample from each treatment were measured daily.

Test protocols and conditions were presented in Hunt *et al.* (2009a), however, a summary is provided here. The 72 h sea urchin (*H. tuberculata*) larval development test endpoint was percent normal development of pluteus larvae. The procedure used was based on methods described in USEPA (1994) and ASTM (1995) and adapted for use with *H. tuberculata* by Doyle *et al.* (2003). Adult sea urchins were collected from Lurline Bay, Sydney, NSW, transported to the laboratory and spawned within 6 h. Spawning was induced by injecting 2 mL of 1 M KCl solution into the peristomal cavity. Females were inverted in a glass bowl of seawater to allow discharge of eggs, which were collected and

stored in filtered fresh salt water (FSW). Sperm from male urchins was collected dry using a pipette to prevent activation (Dinnel *et al.* 1987) and stored at 4^{o}C in a glass vial until required for fertilisation (<1 hour). Viable gametes were selected on the basis of fertilisation success trials and visual examination of gamete maturity. Eggs were fertilised at an egg:sperm ratio of approximately 1:100, and eggs were introduced into the test vials at a rate of 35 eggs/mL. After the 72 h exposure period, buffered formalin was added to each test vessel. One mL of test solution was drawn directly from the bottom of each test vessel and placed in a Sedgwick-Rafter counting chamber. The first 100 larvae were examined and the numbers of normal and abnormal larvae, based on His *et al.* (1999), were recorded.

The 72 h oyster larval development toxicity test was undertaken using larvae of the rock oyster *S. commercialis* based on methods described by USEPA (1996a) and APHA (1998) and adapted for use with *S. commercialis* by Krassoi (1996). This test has been widely used in testing programs within Australia (ANZECC and ARMCANZ 2000; van Dam and Chapman 2001). The test endpoint was the percent normal development (of D-veliger stage) of larvae and is normally conducted over a 48 h period. However, as the testing was conducted outside the normal spawning season, the test exposure period was extended to 72 h to allow at least 70% of embryos to reach the normal D-veliger stage (Widdows 1993). Oysters were obtained from a clean site at Wallis Lake, NSW. Oysters were spawned by gonad stripping, and viable gametes selected on the basis of fertilisation success trials and visual examination of gamete maturity. Eggs were fertilised by adding spermatozoa to the egg suspension so that the final egg:sperm ratio was 1:100. Test vials were inoculated with 500 ± 50 eggs within 2 h of fertilisation at density of 100 eggs/mL. After 72 h exposure, buffered formalin was added to each vessel. One mL of test solution was drawn directly from the bottom of each test vessel and placed in a Sedgwick-Rafter counting chamber. The first 100 oyster larvae were examined and the number of normal and abnormal D-veliger larvae was recorded in accordance with Krassoi (1996).

The 96 h acute toxicity test used juveniles of the polychaete *D. dentata* and was undertaken based on methods described by APHA (1998) and USEPA (1994, 1996b). The

test endpoint was the percent survival of juvenile organisms at 96 hours. Juvenile polychaetes, 3 to 5 months old were purchased from Aquabait Pty Ltd, Dora Creek, NSW. *D. dentata* is abundant along the NSW coastline in shallow sandy environments (Edgar 1997). *D. dentata* has not been used as a test organism previously. Five individuals were randomly selected and introduced into each 1 L jar. Jars were examined every 24 h and numbers of surviving polychaetes recorded.

The 72 h micro algal growth inhibition (cell yield) test using *N. closterium* was based on methods described by USEPA (1996b) and Stauber *et al.* (1994). The test endpoint was the cell yield at 72 h. *N. closterium* is a unicellular estuarine diatom which was initially isolated from Port Hacking and reared in the CSIRO Marine Algal Supply Service (Strain CS-5) in Hobart and is routinely used to assess toxicity in estuarine waters in Australia (Stauber *et al.* 1994; Stauber *et al.* 2000). Organisms were supplied in log growth phase and used in accordance with the standard protocol for the test (Stauber *et al.* 1994). Guillards™ F/2 nutrient stock solutions were added to each test and control treatments to provide nutrients required for micro algal growth. Micro algae used to inoculate the test vessels were concentrated from cultures in log-growth phase by centrifugation, and re-suspended using dilution water. This process was repeated a second time to remove the original culture medium. Density of micro algae was determined using an Improved Neubauer Haemocytometer and test vessels were inoculated with micro algae such that the final concentration at t = 0 h was approximately 10,000 cells/ml. Test vials were incubated for 72 h in a constant temperature cabinet equipped with cool-white fluorescent tubes to provide 5000 ± 500 Lux continuous lighting. At the end of the incubation period, three counts of algal density were made for each replicate and recorded as the number of cells per μL.

The 96 h acute toxicity test using juveniles of the amphipod *A. compressa* was undertaken based on methods described by APHA (1998) and USEPA (1994, 1996b). The test endpoint was the percent survival of juvenile organisms at 96 h. *A. compressa* has previously been used in the assessment of effluent toxicity in the Sydney area (AWT ES&T 1996; Woodworth *et al.* 1999). Juvenile amphipods (approximately 2-5 mm in

length) were collected from Portarlington, Victoria and held in aquaria in the laboratory until required for testing. Five individuals were randomly selected and introduced into each 1 L jar. Jars were examined every 24 h and numbers of surviving amphipods recorded.

The 96 h fish larval imbalance toxicity test was undertaken using the larvae of Australian Bass *(M.novemaculeata)* in accordance with methods based on USEPA (1994), ISO 7346-1, and OECD Method 203. Research with vertebrates in New South Wales is subject to the Animal Research Act (1985) and the testing was performed under an Animal Research Authority. As part of efforts to reduce the number of fish used in toxicity testing under the terms of approval, reference toxicant tests with larval fish are not performed. Larval fish, approximately 6 to 7 mm in length, were obtained from Searle Aquaculture, Wauchope, NSW and were 27 days old at the commencement of the tests, having just absorbed the yolk (pre-flexion larvae). Five individuals were randomly selected and introduced into each 1 L jar. Jars were examined every 24 h and numbers of surviving amphipods recorded. Fish displaying signs of imbalance were removed and euthanized by addition of Aqui-S and immediately placed in a freezer.

MEASUREMENT OF EXPOSURE CONCENTRATIONS

Concentrations of 1,2-dichloroethane and chloroform were measured. Samples were analysed from test vessels at the start and end of testing in accordance with the methodology presented in Hunt *et al.* (2009a). Samples were collected in 40 mL glass vials with airtight Teflon™ lined lids with zero headspace, were preserved with hydrochloric acid immediately and stored at less than 4°C. Samples were extracted using purge and trap methodology (USEPA 5030B) and analysed by Gas Chromatography Mass Spectrometry (GC/MS) utilising a modification of the USEPA Method 8260B for volatile organic compounds (USEPA 1996c). The limit of reporting was 1 µg/L for all analytes, with the exception of vinyl chloride (10 µg/L). Quality control evaluations were undertaken on each sample batch. No analytes were detected in the method blanks. Recoveries for laboratory control samples and matrix spikes were typically between 80% and 120%, and within the accepted criteria. Differences between primary and duplicate

Table 2. Summary of toxicity test conditions for six test organisms (originally presented in Hunt *et al.* 2009a).

Test species	**Sea urchin** *Heliocidaris tuberculate*	**Rock oyster** *Saccostrea commercialis*	**Alga** *Nitzschia closterium*	**Australian Bass** *Macquaria novemaculeata*	**Polychaete** *Diopatra dentata*	**Amphipod** *Allorchestes compressa*
Test type	Static Non-renewal	Static Non-renewal	Static Non-renewal	Semi-static Renewal at 48 hours	Semi-static Renewal at 48 hours	Semi-static Renewal at 48 hours
Test Type	Sub-chronic	Sub-chronic	Chronic	Acute	Acute	Acute
Test duration	72 hours	72 hours	72 hours	96 hours	96 hours	96 hours
Test end-point	Normal pluteus larvae	Larval development to D-veliger stage	Cell yield at 72-h	Imbalance, including survival	Survival	Survival
Test temperature	20 ± 1°C	20 ± 1°C	20 ± 1°C	20 ± 1°C	20 ± 1°C	20 ± 1°C
Test salinity	35 ± 1‰	35 ± 1‰	35 ± 1 ‰	35 ± 1 ‰	35 ± 1 ‰	35 ± 1 ‰
Test chamber	44 mL vial	44 mL vial	44 mL vial	1 L jar	1 L jar	1 L jar
Dissolved Oxygen Content (mg/L)	100.9 – 115.9	100.9 – 115.9	100.9 - 107.4	102.9 - 119.6	96.9 – 104.3	96.9-104.3
pH	7.6 - 8.3	7.6 - 8.3	7.6 - 8.3	7.5 - 8.1	7.7 - 8.1	7.7 - 8.1
Source of test organisms	Field collected, Sydney	Hatchery reared	CSIRO Marine Algal Supply Service	Hatchery reared	Hatchery reared	Field collected, Portarlington

[1] Reference toxicant limits adopted from Hogan *et al.* (2005)

[2] NaDS – Sodium dodecyl sulfate

samples were generally less than 25%, which was considered acceptable (Hunt *et al.* 2009a). Evaluation of exposure concentrations was presented in detail in Hunt *et al.* (2009a). Exposure concentrations were measured at the start (t = 0 hrs) and end of the testing (t = 72 hrs) for vials and at the start (t = 0 hrs) of testing and at the water change for jars (t = 48 hrs). The geometric mean of the start and end concentrations was used to subsequent measured exposure calculations.

Calculation of Toxicity Metrics

Concentrations of VCHs affecting 50% of test organisms (LC50 and EC50 values) were determined by the trimmed Spearman-Karber Method using TOXCALC™ V5.0 (Tidepool™ Scientific Software). No observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values were determined by performing Dunnett's or Steel's Many-One Rank tests, depending on the distribution of the data. EC50 values were calculated using the Trimmed Spearman Karber method.

SSD and Trigger Value Derivation

In the current study, NOEC data from a sufficient number of test species (i.e. ≥5) were available and therefore the SSD method was used. SSDs were derived using the BurrliOZ™ program (Burr Type III distribution) (Campbell *et al.* 2000). The Burr Type III distribution, adopted for use in Australia, New Zealand and South Africa, is a flexible three-parameter distribution that provides good approximations to the commonly used log-logistic, log-normal, log-triangular and Weibull distributions (Shao 1990). The SSD was used to derive TVs that would be protective of 99%, 95%, 90% and 80% of species. If visual assessment of the BurrliOZ™ plots indicates that a distribution other than the selected Burr Type III distribution fits the data better, then the ETX™ and BurrliOZ™ programs, or other appropriate software, should both be used. The fit of the log-normal (ETX™) and Burr Type III (BurrliOZ™) distributions should then be assessed by analysis of the correlation between observed and predicted toxicity for each model, and the best fitting distribution should be adopted.

Toxicity data were manipulated before being used in the derivation of SSDs. Two such manipulations were the classification of data as acute or chronic and the size of the acute to chronic ratio (ACR) used to convert acute data to estimates of chronic toxicity. It is not entirely clear whether the sea urchin and oyster early life stage (ELS) tests are acute or chronic. For example, the Australian and New Zealand WQGs (ANZECC and ARMCANZ, 2000) consider tests with an exposure duration of ≤96 hours to be acute, unless the test organism is a micro-organism, in which case, durations of ≥72 hours are considered chronic (Warne 2001). In contrast, others (e.g. (USEPA 2002; Stauber 2003; Warne 2008) consider ELS test data to be chronic. In this study, oyster and urchin larval development tests were considered to be chronic and no ACR was applied to the NOEC data generated. The default ACR used by ANZECC and ARMCANZ (2000) is 10, however, Di Toro *et al.* (2000) and McGrath *et al.* (2004) found ACRs for non-polar narcotic contaminants to be closer to 5, with estimations of 4.5 ± 2.5 and 5.09 ± 0.95, respectively. In the derivation of each SSD, an ACR of 5 was applied to EC50 values from the polychaete, amphipod and fish tests. No ACR was applied to EC50 values from sub-chronic (i.e. sea urchin and oyster larval development) and chronic (i.e. alga) tests. Where only a LOEC was available, it was converted to a NOEC by dividing by 2.5, in accordance with ANZECC and ARMCANZ (2000).

DATA ANALYSIS

Hobbs (2006) calculated the 95% confidence limits of estimates for the PC95 and PC50 using the BurrliOZ™ software (Campbell *et al.* 2000). As the curve fitting in BurrliOZ™ is a bootstrap technique, confidence intervals (CI) using the same toxicity data will vary with each run. Using the method of Hobbs (2006), 95% CI's for the PC95 and PC50 values were estimated by calculating the 2.5% and 97.5% confidence intervals in BurrliOZ™. The 2.5% and 97.5% intervals were each estimated 10 times and the geometric means of the estimations were used as the lower and upper limits of the 95 % CI. Hobbs (2006) used the non-overlapping of 95% CIs as a criterion to determine significant differences between the PC95 and PC50 values for Australasian and non-Australasian SSDs. The same technique was used in the current study to determine differences between the new TVs derived and the each of the SSDs for the existing ANZECC and ARMCANZ (2000) TVs, based on QSAR generated data with an

application of 10 applied. Hobbs (2006) noted that whilst non-overlapping 95% CIs indicated a significant difference, a statistical test was required to determine if significant differences existed when CIs overlapped. In the present study, a t-test was used to determine significant differences.

RESULTS AND DISCUSSION

TOXICITY TESTING

Exposure concentrations were measured to accurately determine the exposure of test organisms during testing and strong polynomial relationships (r^2>0.99) between nominal and measured exposure concentrations were derived and presented in detail in Hunt *et al.* (2009a). Measured exposure concentrations indicate that the vials were effective in maintaining constant exposure concentrations during testing, with no loss, outside the range of analytical variability identified (Hunt *et al.* 2009a). Jars, however, were less effective in maintaining constant exposure concentrations, with average losses of 52% for 1,2-dichloroethane and 57% for chloroform (Hunt *et al.* 2009a), most probably because of the relatively large head-space.

The quality control criteria were met for each of the tests, including maintenance of water quality parameters (Table 2); survival in controls and reference toxicants (Table 3), with the exceptions identified below. In the larval fish (*M.novemaculeata*) testing, survival in the FSW control was only 53%, less than the control limit of 80%. Survival in the lowest exposure treatments for chloroform and 1,2-dichloroethane were 80% and 87% respectively, meeting the test quality criteria. It is considered that although all control limits were not met, the data is of sufficient quality for inclusion in the assessment of sensitivity and the derivation of TVs. The effect of including or excluding the fish data in the derivation of TVs is investigated further below. The lowest exposure concentration for chloroform for the sea urchin test elicited a significant negative response (reduced normal larval development) and therefore, is a LOEC rather than a NOEC. There was a continually increasing response in the observed toxicity, therefore, there is no reason to question the validity of this data. The effect of including or excluding the sea urchin NOEC on the derivation of TVs will also be investigated further below. Oyster larval development was slow and the test was extended to 72 hours from the original 60 hours to ensure sufficient organisms met the d-veliger larval stage. This was considered to result

from undertaking the testing outside the regular spawning season, which is consistent with the findings of others (Widdows 1993).

For 1,2-dichloroethane, NOECs varied from 580 µg/L (for both the algal and urchin tests) to 159,000 µg/L (for the amphipod test) and for chloroform, NOECs varied from <4µg/L (for the sea urchin test) to 43,100 µg/L (for the polychaete and amphipod tests) (Table 4). Dunnett's Test was used to determine the NOEC and LOEC for all tests with the exception of *H.tuberculata* (sea urchin) in the 1,2-dichloroethane test, where Steel's Many-One Rank test was used. For 1,2-dichloroethane, calculated EC50 values varied from 17,500 µg/L (for the algal test) to 245,000 µg/L (for the amphipod test) and for chloroform varied from 122 µg/L (for the sea urchin test) to 98,800 µg/L (for the oyster test).

The sea urchin larval development, *H. tuberculata*, and the algal growth, *N. closterium*, bioassays were consistently the most sensitive test organisms, whilst the amphipod, (*A. compressa*), and oyster (*S. commercialis*) were the least sensitive tests (Table 4).

ARE EXISTING TVS FOR VCHS PROTECTIVE OF INDIGENOUS SPECIES?

The current TVs for chloroform (770 µg/L) and 1,2-dichloroethane (1900 µg/L) were compared to the toxicity data generated for indigenous species tested in the current study in order to assess whether the TVs were protective. With the exception of the sea urchin and the alga, the NOECs for the oyster and fish larvae, amphipods and polychaete worms were all greater than the PC95 TVs for both chloroform and 1,2-dichloroethane (Table 4). For 1,2-dichloroethane, NOECs for the alga and sea urchin (both 580 µg/L) were approximately one third the magnitude of the PC95 TV (1900 µg/L). For chloroform, the NOEC for the alga (40 µg/L) and sea urchin (4 µg/L) were approximately 20 and 200 fold respectively, less than the TV (770 µg/L). Thus, although the 95% TVs aim to protect 95% of species from chronic effects, in this limited study of indigenous marine organisms, the TVs only protected 66% of species, considerably less than the desired level of protection. It is concluded, based on the available data, that the existing *low reliability* TVs are insufficient in the specified level of protection of sensitive endpoints for marine organisms.

DERIVATION OF NEW TVS FOR CHLOROFORM AND 1,2-DICHLOROETHANE

The results indicate that the existing *low reliability* TVs in ANZECC and ARMCANZ (2000) are not protective of indigenous organisms. Therefore, it is appropriate to derive new higher reliability TVs using toxicity data generated in the current study using the Burr Type III method (using BurrliOZ™), in accordance with the guidance provided in ANZECC and ARMCANZ (2000).

Table 3. Test Acceptance Criteria and survival in controls.

Organism	Test Acceptance Criteria	FSW control	Reference Toxicant Control Limit	Reference Toxicant Result
Alga	Cell yield ≥ 30,000 cells/mL	58 250 cells/mL	19 - 24 µg Cu^{2+}/L[1]	22.7 µg Cu^{2+}/L
Fish	≥80% survival in controls	**53% survival**	Not Applicable	Not Applicable
Polychaete	≥90% survival in controls	100% survival	Not Applicable	Not Applicable
Sea Urchin	≥70% normal larvae in controls	93% normal	7.5-10.1 µg Cu^{2+}/L	9.1 µg Cu^{2+}/L
Oyster	≥70% normal larvae in controls	83% normal	15.1-26.8 µg Cu^{2+}/L	19.8 µg Cu^{2+}/L
Amphipod	≥90% survival in controls	100% survival	0.84-5.4 mg $NaDS^{2}/L$	3.53 mg NaDS/L

Table 4. Summary of NOEC, LOEC and EC50 data for bioassays (in mg/L)

Analyte		Alga	Urchin	Oyster	Fish	Polychaete	Amphipod
1,2-dichloroethane	NOEC	0.58	0.58	121.42	57.65	57.65	159.27
	LOEC	4.95	4.95	369.89	159.27	159.27	430.70
	EC50	17.48	55.77	198.09	73.31	52.60	244.93
Chloroform	NOEC	0.042	<0.004	55.18	15.79	43.18	43.18
	LOEC	0.296	0.004	206.42	43.18	150.81	150.81
	EC50	0.209	0.122	98.81	26.11	80.69	74.84

Although the toxicity testing contained some quality limitations, i.e. low survival in the fish FSW control and no NOEC value being identified in the sea urchin testing for chloroform, it is recommended that these values be included in the derivation of new TVs. Fish survival was below the control limit of 80% in the FSW control (53%) (Table 3), however, each replicate contains only five test organisms and is therefore, sensitive to loss of one organism, resulting in a lower survival rate that meets the acceptance criteria (80%). Fish survival in the lowest concentration treatments of 1,2-dichloroethane and chloroform were 80% and 87% respectively, and therefore, met the control limits. Given this it is argued that the results of the fish toxicity test are valid for use in the derivation of TVs. Previous fish larval imbalance toxicity tests with *M. novemaculeata* used 60 day old larvae (Cohen and Nugegoda 2000), however, the larvae in the current study were 27 days old, in order to meet the requirements for an early life-stage test (USEPA 2002). This difference in age may have influenced survival in the controls. In the sea urchin toxicity testing for chloroform, a NOEC was not identified as the lowest exposure concentration elicited a significant response. As the sea urchin FSW control was within the control limits, the reference toxicant indicated a suitability sensitivity of the test organisms and there was a continually increasing response to the toxicity, there is no reason to exclude the NOEC data from derivation of the SSD. The response at a low concentration merely indicates that the sea urchin larval development test is a sensitivity measure of toxicity and it is considered that inclusion of all six species provides greater representation of the aquatic organisms in the south-eastern region of Australia for the purposes of deriving TVs.

In Australia, the limited range of indigenous test organisms available influences test species selection, endpoints and methods. The lack of available chronic indigenous test organisms has been noted previously (Warne and Westbury 1999; van Dam and Chapman 2001) and the limited number of test organisms and chronic tests available limits the selection of test organisms for a testing program. The available selection of test organisms is dominated by low trophic level organisms and many are laboratory specific and therefore not widely available.

Table 5. Derived SSDs and TVs and existing ANZECC and ARMCANZ (2000) SSDs and TVs (TVs are in µg/L).

SSD Type	Distribution Type	99% TV	95% TV	90% TV	80% TV
Chloroform – new TVs	Burr III	0.01	2	15	100
Chloroform ANZECC and ARMCANZ (2000)	Burr III	370	770	1100	1900
1,2-dichloroethane – new TVs	Log-normal	55	165	550	1450
1,2-dichloroethane ANZECC and ARMCANZ (2000)	Burr III	1000	1900	2600	4000

Guidance is provided in ANZECC and ARMCANZ (2000) and Warne (2001) on the data requirements for derivation of *high*, *moderate* and *low reliability* TVs. It is possible to derive TVs for all three levels of reliability using either the AF method or the SSD method; however, the SSD method is preferred. To derive a *high reliability* TV using the SSD method requires chronic NOEC data for ≥5 species that belong to ≥4 taxonomic groups. If acute toxicity data are available from ≥5 species that belong to ≥4 taxonomic groups that meet the requirements of the SSD method, then a *moderate reliability* TV can be derived (Warne 2001). The new TVs derived in the current study for each of chloroform and 1,2-dichloroethane would be classed as *moderate reliability* as acute toxicity data for ≥5 species is available. A *moderate reliability* classification is higher than the *low reliability* classification applied to the existing TVs. In accordance with the requirements of ANZECC and ARMCANZ (2000), SSDs were derived with all data combined into one dataset. Although Campbell *et al.* (1999) suggested that SSDs may be best derived for different taxonomic groups separately, differences in the sensitivity of different groups are most likely to occur when there are specific modes of action (i.e. for pesticides), however, differences are less likely to occur when there is a non-specific mode of action (Maltby *et al.* 2005), as is the case in the current study.

A Burr Type III distribution was derived for chloroform using the BurrliOZ™ program (Figure 1; Table 5). TVs were derived for 80%, 90%, 95% and 99% protection levels and varied from 0.01 µg/L to 100 µg/L (Table 5). The 95% TV (2 µg/L) is therefore, recommended for slightly to moderately disturbed ecosystems, in accordance with ANZECC and ARMCANZ (2000). For 1,2-dichloroethane, the BurrliOZ™ program plotted a Reciprocal Pareto distribution (Figure 2; Table 5), with TVs varying from 0.5 µg/L to 1 600 µg/L. In some cases, as is the case in the current study, where a suitably accurate Burr Type III distribution cannot be fitted, the BurrliOZ™ program will discard the Burr Type III distribution and fit a reciprocal Weibull or reciprocal Pareto distribution (Campbell *et al.* 2000). Upon visual assessment of the SSDs, the log-normal appeared to be a better fit to the NOEC data and therefore a correlation between the predictions of each of the log-normal and Reciprocal Pareto distributions with the original data was undertaken. The log-normal distribution was a better fit ($r^2 = 0.99$) than the Reciprocal

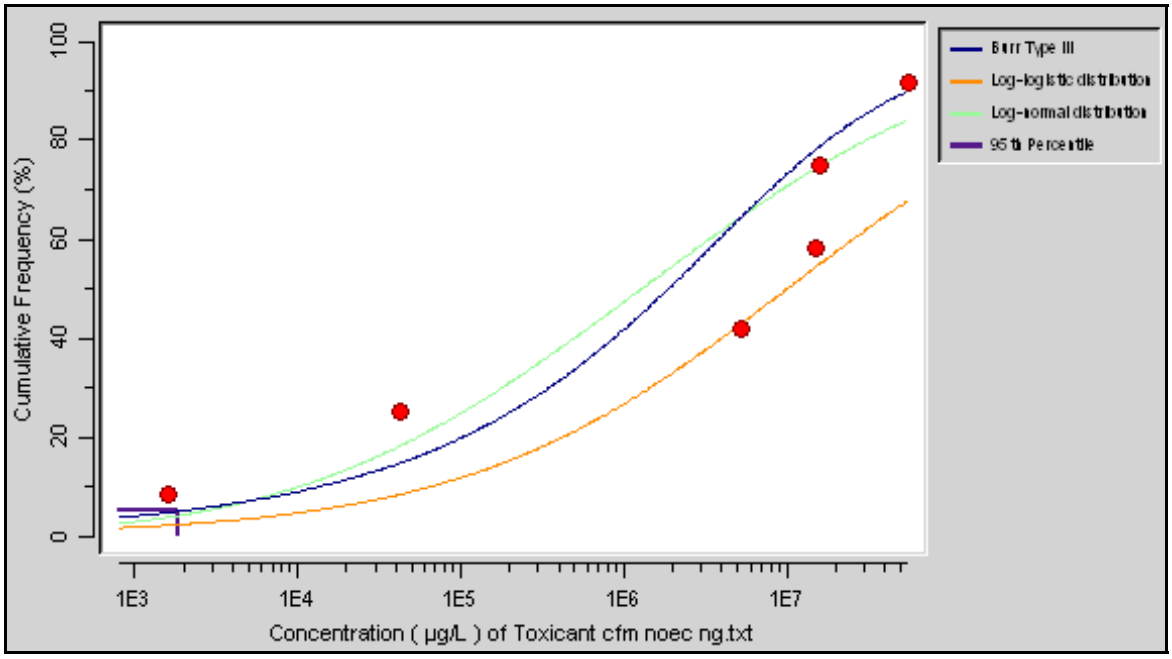


Figure 1. Species sensitivity distributions (SSD) for chloroform including log-normal (green), log-logistic (orange) and Burr type III (blue) distributions.

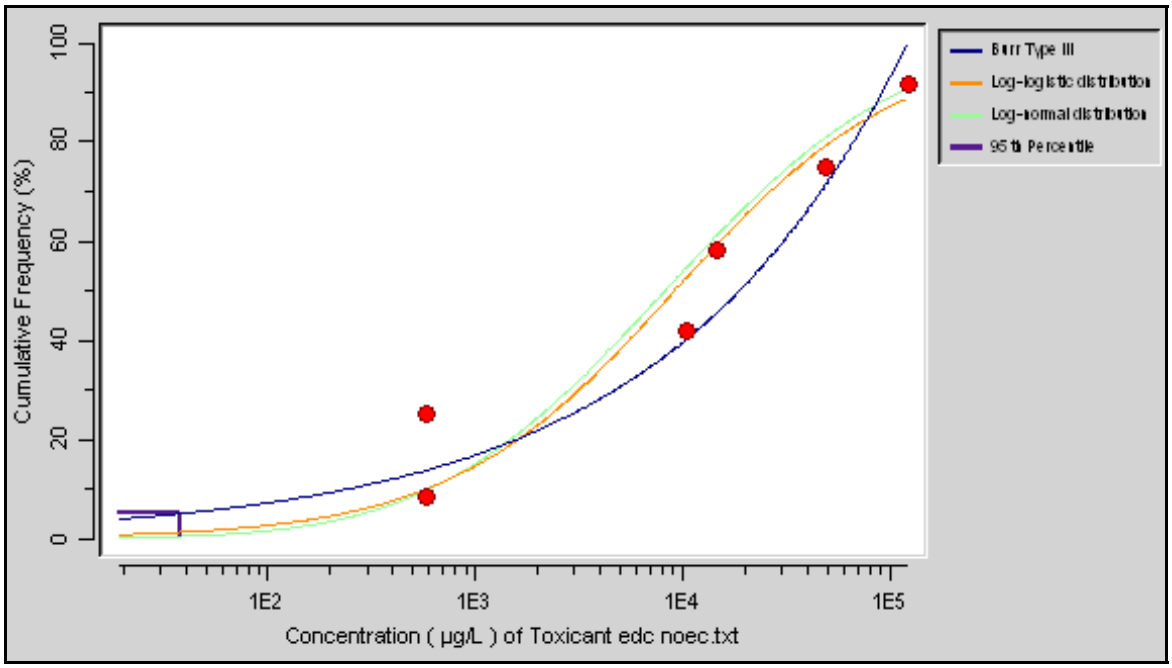


Figure 2. Species sensitivity distributions (SSD) for 1,2-dichloroethane including log-normal (green)(r^2=0.99), log-logistic (orange) and Reciprocal Pareto (blue)(r^2=0.91) distributions.

Pareto distribution ($r^2 = 0.91$). Hunt *et al.* (2009b) argued that selection of the Reciprocal Pareto distribution was inappropriate for narcotic contaminants as it is a finite threshold distribution, more suited to toxicants with a threshold mode of action, such as metals, e.g. copper (Brix *et al.*, 2001) and zinc (van Sprang *et al.*, 2004). The log-normal model, on the other hand, is a continuous distribution more suitable for the toxicants in this study (VCHs), which do not have a threshold mode of action. Given the more appropriate conceptual underpinning of the log-normal distribution and as it was a better fit to the data, it was adopted as the SSD for 1,2-dichloroethane. The 95% TV for the log-normal distribution for 1,2-dichloroethane (n = 6) was 165 µg/L. The other TVs varied from 55 µg/L to 1450 µg/L (Table 5).

The effect of including or excluding the fish and urchin data in the derivation of SSDs and TVs was evaluated for both the chloroform and 1,2-dichloroethane SSDs. When the fish data was excluded from the dataset (n = 5) when deriving a SSD for chloroform, the 95% TV decreased marginally from 2 µg/L to 0.5 µg/L. When the sea urchin data was excluded when deriving a SSD for chloroform (n = 5), however, the 95% TV increased considerably from 2 µg/L to 100 µg/L, reflecting the strong effect of the urchin data on the small dataset. For 1,2-dichloroethane, when the fish data was excluded (n = 5), the 95% TV decreased from 165 µg/L to 90 µg/L (using the log-normal distribution), a considerably more pronounced effect for the 1,2-dichloroethane SSD, than for the chloroform SSD. The variability in TVs when individual data points are included or excluded is one of the limitations when using small datasets to derive TVs.

The TVs derived in the current study for chloroform and 1,2-dichloroethane are considerably less than the existing *low reliability* TVs in ANZECC and ARMCANZ (2000). The new 95% TVs for 1,2-dichloroethane (165 µg/L) and chloroform (2 µg/L) are one and two orders of magnitude less than the existing TVs of 1 900 µg/L and 770 µg/L, respectively. When the 95% CIs of the HC5 for the ANZECC and ARMCANZ dataset and the TV derived in the current study for chloroform (Table 6), there is no overlap between the CIs, with the values of the current study being considerably lower

Table 6. Comparison of estimated values for trigger values (TVs) and 95% confidence intervals (CIs) for newly derived TVs and the existing ANZECC & ARMCANZ (2000) SSD (in µg/L).

PC Values	New TVs	ANZECC & ARMCANZ (2000) TVs
Chloroform HC5	1.88 (0.001 – 7.00)	774 (378 – 1 887)
Chloroform HC50	1 882 (249 – 19 150)	5 070 (3 125 – 9 098)
1,2-dichloroethane HC5	165 (2.09 – 1 167)	1 914 (1 095 – 3 839)
1,2-dichloroethane HC50	8 221 (1 311 – 51 527)	10 007 (6 407 – 18 312)

than those in the ANZECC and ARMCANZ (2000) dataset. There was, however, an overlap when the HC50 for chloroform of each dataset is reviewed (Table 6). Statistical analysis indicated that there was no significant difference ($P \geq 0.05$) between the two datasets for chloroform. When the 95% CIs for both HC5 and HC50 values for 1,2-dichloroethane are examined (Table 6), there is an overlap between the data derived in the current study and the ANZECC and ARMCANZ (2000) dataset. No significant difference ($P \geq 0.05$) was identified between the two datasets for 1,2-dichloroethane. For both 1,2-dichloroethane and chloroform, the ranges of the 95% CIs for data derived in the current study were considerably greater than those of the data used in the derivation of the ANZECC and ARMCANZ (2000) data, possibly a result of the high variability associated with the small dataset in the current study. The low TVs are driven by the sensitivity of the alga and the sea urchin tests. The derivation of new TVs that are lower than the existing ANZECC and ARMCANZ (2000) TVs may result from the inclusion of sensitive endpoints (i.e. larval development or growth) for sensitive test organisms, rather than endpoints those that may only be indicative of narcotic toxicity, at relatively high concentrations.

In Hunt *et al.* (2009b), direct toxicity assessment (DTA) of groundwater contaminated with a complex mixture of VCHs derived a site-specific TV of ~840 µg/L, using the same test organisms as the current study. The dominant component of the complex mixture of VCHs was 1,2-dichloroethane (~90% on a weight basis). The TV derived for the groundwater mixture was what would be expected, based on the mixture containing 1,2-dichloroethane (with a 95% TV of 1 900 µg/L) and various other components including carbon tetrachloride (with a 95% TV of 240 µg/L), chloroform (with a 95% TV of 770 µg/L) and vinyl chloride (with a 95% TV of 100 µg/L). The predicted 95% TV, on a toxic unit basis from the existing TVs, was 1 700 µg/L, double the derived TV of 840 µg/L. The TVs derived in the current study for 1,2-dichloroethane and chloroform are considerably lower than TVs derived when a complex, additive mixture of VCHs was present. This indicates that deriving SSDs and TVs with only 5 or 6 species may lead to variability in derived values, similar to the finding of Hose *et al.* (2005). Reliance on a small number of

test species may have a considerable effect on the derived TVs and various levels of protection (i.e. PC95, PC80).

SSDs in the current study exhibited a higher degree of variability than those used in the derivation of the ANZECC and ARMCANZ (2000) TVs, which is likely, in part to be a product of the small dataset. However, the toxicity data in the current study and the derived SSDs, particularly HC5 values, were between one and two orders of magnitude less than the existing TVs, which is consistent with other studies of the toxicity of VCHs undertaken in sealed containers. Tsai and Chen (2007) found that toxicity testing for volatile narcotic contaminants undertaken in open containers underestimated toxicity to algae by up two orders of magnitude, when compared to testing in closed systems. Tsai and Chen (2007) also found that when risk assessments of chemicals were reviewed, approximately 30% resulted in a stricter classification when the testing was undertaken in sealed containers. In a similar study, Chen and Lin (2005) concluded that the toxicity data derived for volatile organic chemicals using standard toxicity testing methods (i.e. not sealed), may underestimate the impact of the chemicals. It is considered unlikely, given the wide variety of non-volatile toxicants tested in the derivation of the QSAR used in the derivation of the ANZECC and ARMCANZ (2000) TVs (van Leeuwen *et al.* 1992), that the toxicity testing was undertaken in sealed containers. The endpoints used in the current study may also be more sensitive than the variety used in the derivation of the QSAR (van Leeuwen *et al.* 1992). Because of these factors, the predicted toxicity estimated by the QSAR is likely to underestimate toxicity of VCHs. Due to the potential underestimation of toxicity in the derivation of the QSAR and dependent TVs, and as the toxicity testing undertaken in the current study, consistent with the research of others (e.g. (Chen and Lin 2005; Tsai and Chen 2007), who identified toxicity at concentrations between one and two orders of magnitude less than the existing TVs, the new *moderate reliability* TVs derived in the current study should be adopted.

The current study identified an overestimation of the degree of protection provided by the existing ANZECC and ARMCANZ (2000) TVs for two VCHs; 1,2-dichloroethane and

chloroform. New and significantly lower TVs were derived in the current study, however, as the TVs for at least twelve other VCHs have been derived using the same QSAR (Warne 2001; Hunt *et al.* 2007), evaluation of the protectiveness of TVs for other VCHs, is required. This lack of protection may extend to more organisms than predicted using the current TVs, however, it also may extend to more sensitive endpoints, for example growth and development, than the survival predominantly used in the derivation of the QSAR and the TVs. As new data become available, re-evaluation of the TVs derived in this study should be undertaken.

CONCLUSIONS

The results indicate that the existing Australian and New Zealand chloroform and 1,2-dichloroethane trigger values (TVs) for slightly to moderately modified ecosystems do not provide adequate protection for indigenous marine organisms protecting 66% rather than 95% of species. The study generated toxicity data for six indigenous marine organisms which permit the generation of *moderate reliability* TVs for 1,2-dichloroethane and chloroform in marine ecosystems in accordance with the framework set out in ANZECC and ARMCANZ (2000). The resulting TVs derived were between 1 and 3 orders of magnitude lower than the existing ANZECC and ARMCANZ (2000) *low reliability* TVs. The derivation of TVs in this study may be dependent on the small number of test organisms and the selection of sensitive test endpoints used in the derivation. Further testing of volatile chlorinated hydrocarbons is needed to support both this study and the existing guidelines. Further development of indigenous chronic bioassays is urgently required.

PAPER 7

QUANTIFYING REDUCTION IN ECOLOGICAL RISK IN PENRHYN ESTUARY, SYDNEY, FOLLOWING GROUNDWATER REMEDIATION

ABSTRACT

The environmental risk associated with discharge of contaminated groundwater containing a complex mixture of at least 14 volatile chlorinated hydrocarbons (VCHs) to Penrhyn Estuary, Sydney (Australia) has been previously assessed. The probabilistic ecological risk assessment (ERA) was undertaken using surface water monitoring data from 2004 to 2005; however, a groundwater remediation system was installed in 2006 to prevent further discharge of VCHs into the estuary. Following the installation of the remediation system, the ecological risk has not been assessed. The present study assessed ecological risk following implementation of the groundwater remediation system to evaluate the success of the project. The risk assessment was undertaken using a toxicity distribution derived from direct toxicity assessment of the contaminated groundwater, exposure data from surface water monitoring between 2007 and 2008 and the joint probability curve (JPC) methodology. Following implementation of the remediation system, ecological risk decreased by up to two orders of magnitude in source areas, i. e. from a maximum risk (δ) of 84% to <1% in Springvale Drain source area. In Penrhyn Estuary, risk decreased by up to one order of magnitude, from a maximum of 36% to ~1.4% in Springvale Tributary. Following remediation, ecological risk (δ) in Penrhyn Estuary decreased to less than 1% in all other locations within the estuary irrespective of the tide and >95% of species are protected >95% of the time.

INTRODUCTION

A quantitative probabilistic ecological risk assessment of groundwater contamination, undertaken in Penrhyn Estuary, Sydney, Australia, identified unacceptable risks to aquatic organisms resulting from exposure to volatile chlorinated hydrocarbons (VCHs)(Hunt *et al. in press a*). These authors undertook a probabilistic ecological risk assessment (ERA) using an exposure distribution derived from surface water monitoring in the estuary and a toxicity distribution derived from direct toxicity assessment (DTA) of groundwater from the site using indigenous marine organisms (Hunt *et al.* 2009b). Ecological risk was characterised using the joint probability curve (JPC) approach (Hunt et al., *in press a*), for which the area under the curve (δ) is equal to the degree of overlap of the exposure and toxicity distributions (Solomon *et al.* 2000). Although more data are generally required, a key advantage is that use of distributions for exposure and toxicity over point estimates allows quantitative estimation of risk (Solomon and Takacs 2002) and can incorporate variability and uncertainty into risk estimates (Roberts 1999). JPCs, for example as used in Hunt et al., (*in press a*), display the magnitude of effect on the *x* axis and the frequency (or probability) on the *y* axis. As an SSD (with proportion of species affected and concentration) and exposure probability plot (with concentration and probability) have a common axis, i. e. the concentration of contaminant, they can be rationalised into a single plot, the JPC with two axes – probability and proportion of species affected. The area under the JPC curve has been shown to be mathematically equivalent to the overlap of the exposure and toxicity curves (Aldenberg *et al.* 2002; van Straalen 2002). The shape of the curve can be also be used to define acceptable or unacceptable ecological risks, providing an indication of the type and duration of the exposure.

Whereas risk assessments are commonly undertaken to assess the current or predicted ecological risk associated with contamination, these assessments are not often revisited after remediation. Monitoring and feedback of changes in risk is vital to the success of any risk assessment and is central to the risk assessment framework. The value of risk assessment becomes limited if conditions change and the risk assessment is not updated (Burgman 2005). The current study revisits an earlier risk assessment to update the original evaluation to evaluate the change in conditions.

PROBLEM FORMULATION

The problem formulation phase was originally provided in the screening level hazard assessment (Hunt et al. 2007), however, a summary is provided. Penrhyn Estuary is a small (10 ha), tidal embayment located approximately 10 km south of the Sydney central business district on the northern shoreline of Botany Bay, New South Wales (NSW), Australia (Figure 1). Land use in the 320 ha catchment includes residential, commercial and both light and heavy industrial. The intertidal embayment is inundated at high tide, with water covering an area of approximately 4.0 ha, whereas at low tide, mudflats are exposed and the area covered by water is approximately 0.4 ha. The estuary was originally devoid of vegetation when it was formed in the late 1970s using sandy dredge spoil from development of the adjacent port however, today it supports a variety of flora species, including mangroves, saltmarsh species and dune vegetation and also attracts wading shorebirds, which forage on the mudflats at low tide. The fauna and flora are typical of that found in south eastern Australian marine and estuarine environments (Edgar 1997).

The VCHs in the present study are characterised by high water solubility and low octanol-water partition coefficients (K_{ow}) (i.e. less than 3), indicating low potential for bioaccumulation (Carey *et al.* 1998). Direct exposure to VCHs in the water column was identified as the likely primary source of uptake (Hunt et al. 2007). VCHs act under the non-polar narcotic mode of action (McCarty and Mackay 1993a; Carey *et al.* 1998). The toxicity of groundwater that discharges into the estuary to aquatic receptors was evaluated by Hunt et al. (*in press b*).

The previous risk assessment classified the estuary into 4 areas: the Springvale Tributary (SVT); the Floodvale Tributary (FVT); the inner estuary (IE); and the outer estuary (OE) (Figure 1). This earlier assessment (Hunt *et al. in press a*) identified risks of up to 84% in the source areas, with risks of up to 36% identified in the Springvale Tributary and up to 9.5% in the Inner Estuary. The assessment ranked risk as low tide > both tides > high tide. The risk was also identified as being greatest in the SVT > IE > FVT > OE. The key

process reducing concentrations of VCHs in the estuary was identified as dilution with seawater entering from Botany Bay (URS 2005).

Discharge of groundwater containing VCHs to the estuary occurred from the early 1990s until 2005, when the groundwater treatment plant (GTP) was commissioned (Stening et al. 2008). The GTP is a 'pump and treat' groundwater system that extracts groundwater from a network of 113 wells and has a capacity to extract and treat up to 15ML d^{-1} of high quality water for re-use. During the treatment process VCHs are stripped from the groundwater and destroyed at high temperature, using a Thermal Oxidation unit (Stening et al. 2008). As a consequence of the operation of the GTP, the discharge of groundwater contaminated with VCHs to the stormwater drains in the Springvale and Floodvale Drain source areas decreased, resulting in a reduced load of VCHs to Penrhyn Estuary. The GTP achieved its objective of 'hydraulic containment' of the groundwater (Stening et al. 2008). Success of the remediation project has, however, only been measured in terms of engineering (i. e. successful construction and operation) and chemical (i.e. lower concentrations of VCHs) criteria. However, the risk assessment has not been re-visited to evaluate whether the project has achieved the overall objective of decreasing the ecological risk to organisms resident in the estuary (i.e. relating chemical concentrations to potential for toxicity). Measurement of chemical concentrations alone does not provide quantitative information on ecological risk, merely qualitative information that risk should decrease. Monitoring of the implications of management actions on risk is vital to the success of any risk assessment and is central to the risk assessment framework (Suter, 1993; NEPC, 1999). As the overall objective is to protect aquatic organisms in Penrhyn Estuary and ultimately, Botany Bay, it is imperative that the 'success' of the project be measured in terms of ecological risk. The objective of the present study was, therefore, to revisit the risk assessment for VCH contamination of the estuary to assess the changed conditions and quantify the reduction in ecological risk following implementation of the groundwater remediation program and thereby, measure success of the project.

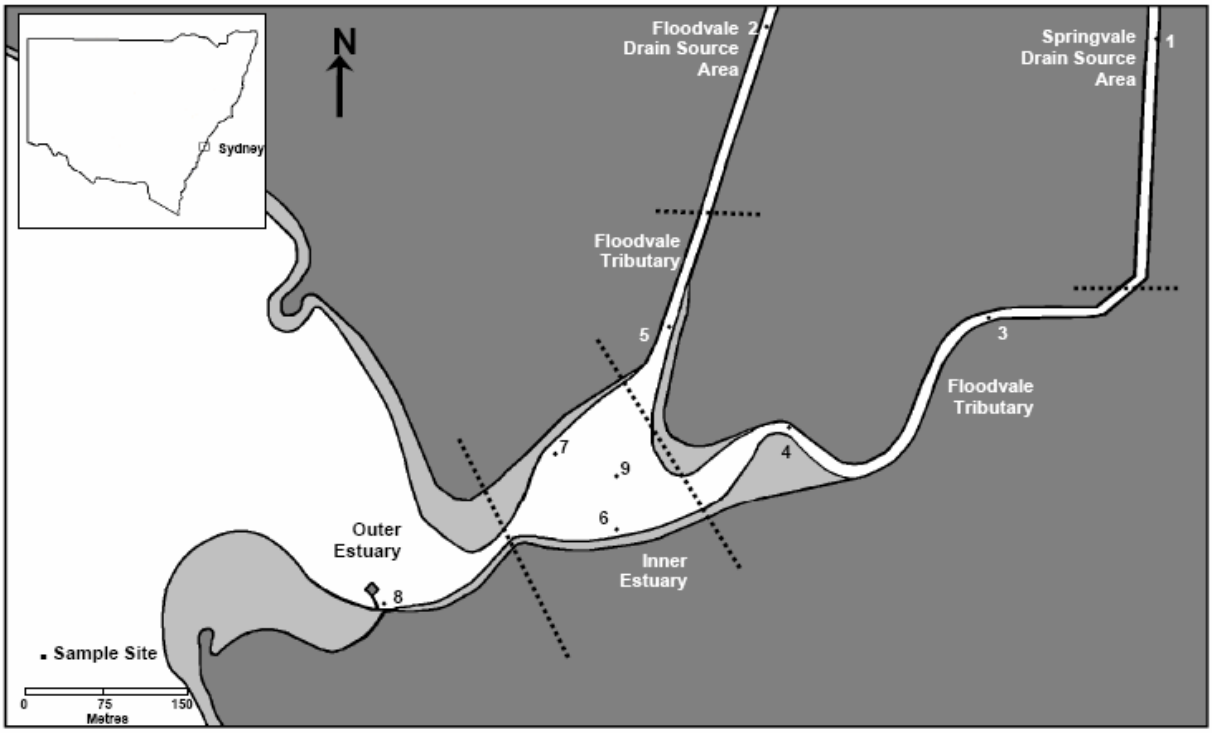


Figure 1. Sample locations within Penrhyn Estuary. Dashed lines denoted the various areas within the estuary.

METHODOLOGY

EXPOSURE ASSESSMENT

To characterise exposure to VCHs in surface water in the estuary, seven sites were selected with two sites located in Springvale Tributary; one site in the Floodvale Tributary; three sites in the inner estuary; and one site in the outer estuary (Figure 1). Samples were collected from the concrete-lined stormwater drain at the head of Springvale Drain and Floodvale Drain in the source areas; however, as these drains do not constitute ecosystems, they have not been assessed for ecological risk *per se*, but were included for comparative purposes and source characterisation. Sample sites were consistent between pre-remediation and post-remediation monitoring programs.

To characterise exposure to concentrations of VCHs in surface water in the estuary prior to the remediation, sampling of estuarine water and analysis for VCHs was undertaken over a one-year period (in 2004 and 2005) in two monitoring programs as detailed in Hunt et al. (2007, *in press a*). Samples were collected at high- and low-tides and the programs aimed to characterise short- and long-term variability in VCH concentrations. Data were compiled from all sites at high- and low tides and used to quantify three exposure scenarios: (1) the average of aqueous VCH concentrations at both high and low tides, (2) high tide VCH concentrations only, and (3) low tide VCH concentrations only. The three exposure scenarios were determined within the four areas of the estuary: Springvale Tributary; the Floodvale Tributary; the Inner Estuary; and the Outer Estuary.

After the commencement of operation of the groundwater treatment plant (GTP), surface water samples were collected every three months from March 2007 to March 2008, to characterise concentrations of VCHs. Data were compiled for the same three exposure scenarios and the same four areas as the pre-remediation ERA (*in press c*). Six samples were available from each of the four areas for assessment at low- and high tides, with 12 samples available across both tides.

Samples were collected in 40 mL glass vials with airtight Teflon™ lined lids with zero headspace, preserved with hydrochloric acid and immediately stored at less than 4°C. Samples were extracted using purge and trap methodology (USEPA 5030B) and analysed by Gas Chromatography Mass Spectrometry (GC/MS) utilising a modification of the USEPA Method 8260B for volatile organic compounds (USEPA 1996c) as described in the DTA (Hunt *et al.* 2009b). The limit of reporting was 1 µg/L for all analytes with the exception of vinyl chloride (10 µg/L). Quality control evaluations were undertaken on each of the sample batches and no analytes were detected in the method blanks. Recoveries for laboratory control samples and matrix spikes were between 80% and 120%, and within the acceptable criteria. Differences between primary and duplicate samples were generally less than 25%, typical of the variability observed between duplicate samples for these contaminants at this laboratory and considered acceptable (Hunt *et al.* 2007; URS 2008; Hunt *et al. in press a*). Values that were less than the limit of reporting (LOR) were assigned a concentration equal to half the LOR, considered a conservative approach (Warren Hicks *et al.* 2002), consistent with the previous risk assessment. The distributions of each of the exposure datasets, for three tidal exposures in each of the four areas, were assessed for log-normality using the Anderson-Darling test.

EFFECTS ASSESSMENT

All the data required for the post-remediation effects assessment was obtained from the pre-remediation effects assessment (Hunt *et al*. 2009b) and details are provided there. This is appropriate as the sensitivity of the test organisms will not have changed between pre- and post-remediation, however, the concentrations of VCHs to which they are exposed may well have changed. Direct toxicity assessment (DTA) of the contaminated groundwater was undertaken on five species (a 72 hour algal (*Nitzschia closterium*) population growth test; a 72 hour sea urchin (*Heliocidaris tuberculata*) larval development test; a 72 hour oyster (*Saccostrea commercialis*) larval development test; a 96 hr amphipod (*Allorchestes compressa*) survival test; and a 96 hr juvenile polychaete (*Diopatra dentata*) survival test) that belong to four taxonomic groups and thus meet the minimum data requirements for deriving SSDs (ANZECC and ARMCANZ 2000; Warne 2001). The species were considered representative of the receiving environment, ecologically relevant

and with some commercial or recreational value in the area. To prevent loss of volatile contaminants and potential underestimation of toxicity, toxicity testing was undertaken in sealed containers, using the methodology in Hunt *et al*. (2009a).

In the derivation of the SSD, an acute to chronic ratio (ACR) of 5 was applied to NOECs from the polychaete and amphipod tests, in accordance with the estimations of 4.5 ± 2.5 (McGrath *et al.* 2004) and 5.09 ± 0.95 (Di Toro *et al.* 2000). The two larval development tests for the urchin and oyster (sub-chronic tests), were treated as chronic tests for the purposes of guideline derivation and no ACR was applied. The effect of selection of ACR and treatment of sub-chronic tests as chronic tests is evaluated in Hunt et al. (2009b).

Hunt *et al*. (*in press b*) compared the fit of the Reciprocal Pareto, (the distribution determined by the software used to derive the Australian and New Zealand water quality guidelines i.e. BurrliOZ™ (Campbell *et al*., 2000), and log-normal statistical distributions to the pre-remediation toxicity data of the five test species to total VCH concentration and concluded that the log-normal gave the best fit to the data. The log-normal distribution was, therefore, adopted in the current study. The use of an SSD derived using NOEC data in both pre- and post-remediation ERAs was considered to provide a conservative estimate of ecological risk, based on the loss of protection of organisms and characterising the risk of potential adverse effects occurring. This is consistent with objective of the remediation, i. e. to achieve protection of aquatic organisms in the receiving ecosystem.

RISK CHARACTERISATION

Hunt *et al*. (*in press a*) identified the multiple benefits of using the JPC methodology to characterise risk in the estuary, including the quantification of the risk (i.e. δ, the area under the curve) and provision of information on the type of exposure (i.e. the shape of the curve). Risk values (δ) and JPCs were estimated using the ETX™ program (van Vlaardingan *et al*., 2004), which estimates log-normal curves for each of the exposure and toxicity distributions and the extent of overlap between these two distributions. Risk was characterised for the three exposures scenarios (i.e. data across both tides and high- and

low tides alone) using NOEC toxicity data for each of the four areas (Springvale and Floodvale Tributaries and the inner and outer estuaries), resulting in a total of 12 risk values. Where standard deviations of exposure data were too large for ETX™ to calculate an area under the curve (AUC), these were estimated manually. The previous risk assessment (Hunt *et al*. *in press a*) used a threshold of 5% of acceptable risk, consistent with the inherent assumptions in the ANZECC and ARMCANZ (2000) Guidelines, which were also adopted in the current study.

RESULTS AND DISCUSSION

EXPOSURE ASSESSMENT

The mean concentration of VCHs in Springvale Drain source area decreased by approximately two orders of magnitude, from 22 036 µg/L to 218 µg/L (Table 1), following commissioning of the GTP. In Floodvale Drain source area, the mean concentration of VCHs decreased by one order of magnitude, from 1 420 µg/L to 107 µg/L following remediation. In the estuary, mean concentrations post-remediation were 3- (in the Outer Estuary at high tide) to 40-fold (in the Inner Estuary at low tide) lower in ~92% of locations and exposure scenarios, with the one exception being in Floodvale Tributary at high tide, where the mean concentration of VCHs increased from 132 µg/L (pre-remediation) to 177 µg/L (post-remediation). This 'increase' is possibly anomalous and may reflect the high variability in the data from this location (mean = 177 µg/L and standard deviation = 411 µg/L), attributable to minor fluctuations in the concentrations of VCHs in groundwater discharging to the tributary.

Exposure concentrations generally followed a similar trend to pre-remediation exposure concentrations, i. e. concentrations were generally ranked low tide > both tides > high tide. When assessed spatially, pre-remediation exposure concentrations generally decreased in the order Springvale Tributary > Floodvale Tributary ~ Inner Estuary > Outer Estuary, whereas post-remediation, they generally decreased in the order Springvale Tributary > Floodvale Tributary > Inner Estuary > Outer Estuary (Table1), possibly reflecting the much larger decrease in the Inner Estuary compared to the Floodvale Tributary post-remediation.

When log-normality of exposure distributions was assessed using the Anderson Darling test, 50% of exposure scenarios failed (at $P<0.05$). Scenarios that failed were the three tidal scenarios in Floodvale Tributary, the inner and outer estuaries, where large number of samples recorded concentrations of VCHs that were less than the limit of reporting. This was the case for up to 75% of values in the inner and outer estuary, resulting in a right-

skewed distribution. A lack of log-normality in exposure distributions occurred for 25% of exposure scenarios in the previous ERA (Hunt *et al. in press a*). The increased frequency of failing the test log-normality from 25% to 50% may reflect the lower levels of contamination in the estuary and greater frequency of values less than the limit of reporting, following remediation. The implication of the data failing to fit the log-normal distribution is unknown, however, it has been suggested by Newman *et al.* (2000b) that violation of this assumption, whilst undesirable, may have little effect on the resulting interpretation of risk.

EFFECTS ASSESSMENT

Toxicity metrics, including NOEC, LOEC and EC50 were derived for the DTA tests in Hunt *et al*. (2007) and expressed in terms of concentrations of total VCHs (Table 2). NOECs varied from 1.11 mg/L for urchin larval development test to 45.5 mg/L for the amphipod survival test. Similar variations in toxicity occurred for the LOEC and EC50 data (Table2). Data were log-normally distributed for the toxicity scenario when assessed using the Anderson-Darling test ($P<0.05$) concentration that should protect 95% of species from experiencing toxic effects (i.e. PC95, which is the equivalent of the concentration that should permit only 5% of species to experience toxic effects - HC5), based on the log-normal SSD was 830 µg/L.

Table 1. Mean concentrations (and standard deviations) of volatile chlorinated hydrocarbons (in µg/L) in surface water in Penrhyn Estuary a) prior to and b) following remediation.

Table 1a)

	Springvale Drain		Floodvale Drain		Springvale Tributary		Floodvale Tributary		Inner Estuary		Outer Estuary	
	Mean	**St.Dev**	**Mean**	**St.Dev**	**Mean**	**St.Dev**	**Mean**	**St.Dev**	**Mean**	**St.Dev**	**Mean**	**St.Dev**
Both Tides	22036	18865	1420	685	1816	2014	329	248	298	671	51.2	92.3
High Tide	--	--	--	--	1363	2436	132	146	91.5	83.3	31.8	42.6
Low Tide	--	--	--	--	2273	2098	419	141	669	1013	151	161

Table 1b)

	Springvale Drain		Floodvale Drain		Springvale Tributary		Floodvale Tributary		Inner Estuary		Outer Estuary	
	Mean	**St Dev**	**Mean**	**St Dev**	**Mean**	**St Dev**	**Mean**	**St Dev**	**Mean**	**St Dev**	**Mean**	**St Dev**
Both Tides	218	195	107	172	164	149	94.9	290	13.4	15.9	9.04	7.53
High Tide	--	--	--	--	136	118	177	411	9.08	7.55	9.83	9.64
Low Tide	--	--	--	--	191	183	12.4	6.87	17.7	21.3	8.25	5.52

-- denotes that no values were derived as the area is not tidal

Table 2. Summary of NOEC, LOEC and EC50 metrics for toxicity testing of marine test organisms exposed to groundwater contaminated with volatile chlorinated hydrocarbons. (Note: acute to chronic ratios have not been applied). Data were originally presented in Hunt et al. (*in press-a*).

Toxicity Metric	Alga	Urchin	Oyster	Polychaete	Amphipod
NOEC	2.30	1.11	4.98	29.9	45.5
LOEC	4.98	2.30	10.3	45.5	45.5
EC50	4.10	3.77	9.79	32.1	>45.5

Table 3. Risk values (%) before (3a) and after (3b) implementation of the groundwater remediation system.

Table 3a)	Springvale Drain	Floodvale Drain	Springvale Tributary	Floodvale Tributary	Inner Estuary	Outer Estuary
Both	83.8	15.9	25.0	2.6	4.1	0.1
High	--	--	*16.0*	0.1	0.1	0.0
Low	--	--	35.4	2.3	9.3	0.8

Table 3b)	Springvale Drain	Floodvale Drain	Springvale Tributary	Floodvale Tributary	Inner Estuary	Outer Estuary
Both	0.36	0.35	0.79	0.04	0.00	0.00
High	--	--	0.67	*0.10*	0.00	0.00
Low	--	--	1.33	0.00	0.00	0.00

-- no risk value was derived as these locations are not tidal.

RISK CHARACTERISATION

Using the JPC approach, AUCs were derived for each of the three exposure scenarios (i.e. average concentration tides, high tide concentration and low tide concentration), for each of the four estuary areas (Springvale and Floodvale Tributaries and the Inner and Outer Estuaries), resulting in a total of 12 values for risk (δ) pre- and post-remediation (Tables 3a and 3b, respectively). Risk (δ) in Floodvale Tributary at high tide was assessed manually as the standard deviation was too large for the ETX™ software to calculate. Environmental risk, measured as δ, was lower in all locations and all tidal scenarios following commissioning of the GTP, except for the high tide scenario in the Outer Estuary, where the risk did not change (i.e. it remained 0).

CHANGE IN ECOLOGICAL RISK

In the Springvale Drain source area, risk decreased from approximately 84% prior to the remediation, to <1% post-remediation. In Floodvale Drain source area, risk decreased from approximately 16% to <1% (Tables 3a and 3b). In the estuary, following remediation, the mean risk value (δ) across the four locations and both tides, decreased from 8% to <1%. Decreases in risk of ~30 fold were identified in Springvale Tributary in each tidal scenario, with a decrease of ~60 fold in the Floodvale Tributary when both tides were assessed. In the inner and outer estuary, risk decreased to 0% in all tidal exposure scenarios following remediation. Decreases in the magnitude of risks in each location demonstrate the strong positive impact of the groundwater treatment system on concentrations of VCHs discharging to Penrhyn Estuary. The JPCs also exhibit the strong effect of the remediation on the risk profile, with JPCs after remediation representing acceptable risk profiles, as defined by Solomon and Takacs (2002) (i.e. a small area under the curve). A similar response was identified in each of the areas in the estuary, however, JPCs and a discussion of a selection of the areas is included below. The JPC for Springvale Drain source area (Figure 2a) and Springvale Tributary (Figure 2c) demonstrate a transition from unacceptable high-risk profiles prior to remediation to acceptable lower-risk profiles (Figures 2b and 2d, respectively) following remediation. In the Inner Estuary, risk decreased from a relatively low-risk profile to zero risk, with the JPC indicating that 0% of species were affected 100% of the time following remediation (Figures 2e and 2f, respectively). Environmental risk in the estuary posed by VCHs, following remediation,

decreased to <1% in all scenarios and locations, with the exception of the Springvale Tributary at low tide, where the risk was 1.4%. The ecological implication of the remediation can be assessed directly from the JPCs. In the Springvale Drain source area, pre-remediation, <5% of species would be protected 50% of the time (Figure 2a) however, post-remediation >95% of species would be protected >95% of the time (Figure 2b). In the Springvale Tributary >90% of species would be protected ~50% of the time (Figure 2c); however, post-remediation >95% of species would be protected >95% of the time (Figure 2d). In the inner estuary, the risk profile indicated a change from 90% of species being protected 90% of the time pre-remediation (Figure 2e) to all species being protected all of the time (Figure 2f).

SPATIAL INTERPRETATION OF RISK

Although large differences in the magnitude of risk were identified following the remediation, only minor differences were identified in the spatial distribution of risk within the estuary. Prior to remediation, the risk in the estuary decreased from the greatest risk in Springvale Tributary >> Floodvale Tributary ~ Inner Estuary > Outer Estuary; however, post-remediation, the risk was greatest in Springvale Tributary >> Floodvale Tributary > Inner Estuary = Outer Estuary. Prior to the remediation, Hunt et al (*in press a*) hypothesised that the greater risk in the Inner Estuary reflected the greater input of contaminants from the Springvale Drain source area. Following groundwater remediation, a greater decrease in risk was identified in the Springvale Drain source area than the Floodvale Drain source area (Table 3a and 3b). The larger decrease in risk in the Inner Estuary than in Floodvale Tributary due to the remediation may reflect the greater influence of the Springvale Tributary on the magnitude of contamination in the estuary, than Floodvale Tributary. Overall, whereas the magnitude of environmental risk has decreased in the estuary, the spatial trend in risk following remediation remains similar to that prior to remediation, which reflects the underlying physical characteristics of the source areas and their interaction with Penrhyn Estuary and Botany Bay.

DIFFERENCES BETWEEN EXPOSURE ASSESSMENT SCENARIOS

Prior to remediation, the greatest mean ecological risk was at low tide (mean $\delta = 12$, n=12), followed by both tides (mean $\delta = 8.0$, n=12), with the lowest risk reported at high tide (mean $\delta = 4.0$, n=12) (Hunt *et al. in press a*). Following remediation, the greatest mean ecological risk was at low tide (mean $\delta = 0.34$, n=4), followed by both tides (mean $\delta = 0.21$, n=4), with the lowest risk identified at high tide (mean $\delta = 0.20$, n=4). These interpretations of risk are consistent with the physical characteristics of the estuary, where concentrations of VCHs and risk are highest at low tide and lowest at high tide and reflect the overall pattern reported in the screening level risk assessment where risk at low tide > both tides > high tide (Hunt et al. *2007*) and tidal interaction between the upper estuary and Botany Bay.

IMPLICATIONS OF THE CHANGE IN RISK

The risk values after remediation were considerably less than the 5% threshold level of acceptability for risk, accepted whenever HC5/PC95 values are used and indicate that the remediation system has successfully reduced the magnitude of ecological risk in the estuary to an acceptable level. Together with the risk values, the JPCs also reflect the strong positive impact of the GTP on VCH contamination in the estuary and suggest that VCH contamination has decreased to an acceptably low level, where recovery of the aquatic ecosystem would be expected, provided that there are no other pollutants in the water in Penrhyn Estuary.

The assessment of risk following remediation demonstrated the reduction in risk to acceptable levels. Measurement of concentrations of VCHs alone did not provide quantitative estimates of risk, merely quantitative information that the risk should decline. Re-assessment of the risk assessment is vital to the success of the ERA process and a central feature of the risk assessment framework. Ongoing monitoring of the risk should be undertaken, however, as the sensitivity of test organisms is unlikely to change, chemical analysis of VCHs should be sufficient, when coupled with the existing toxicity distribution, to assess risk in the estuary. However, should the composition of VCHs in the groundwater change, increase dramatically or should new contaminants be identified, additional DTA should be undertaken.

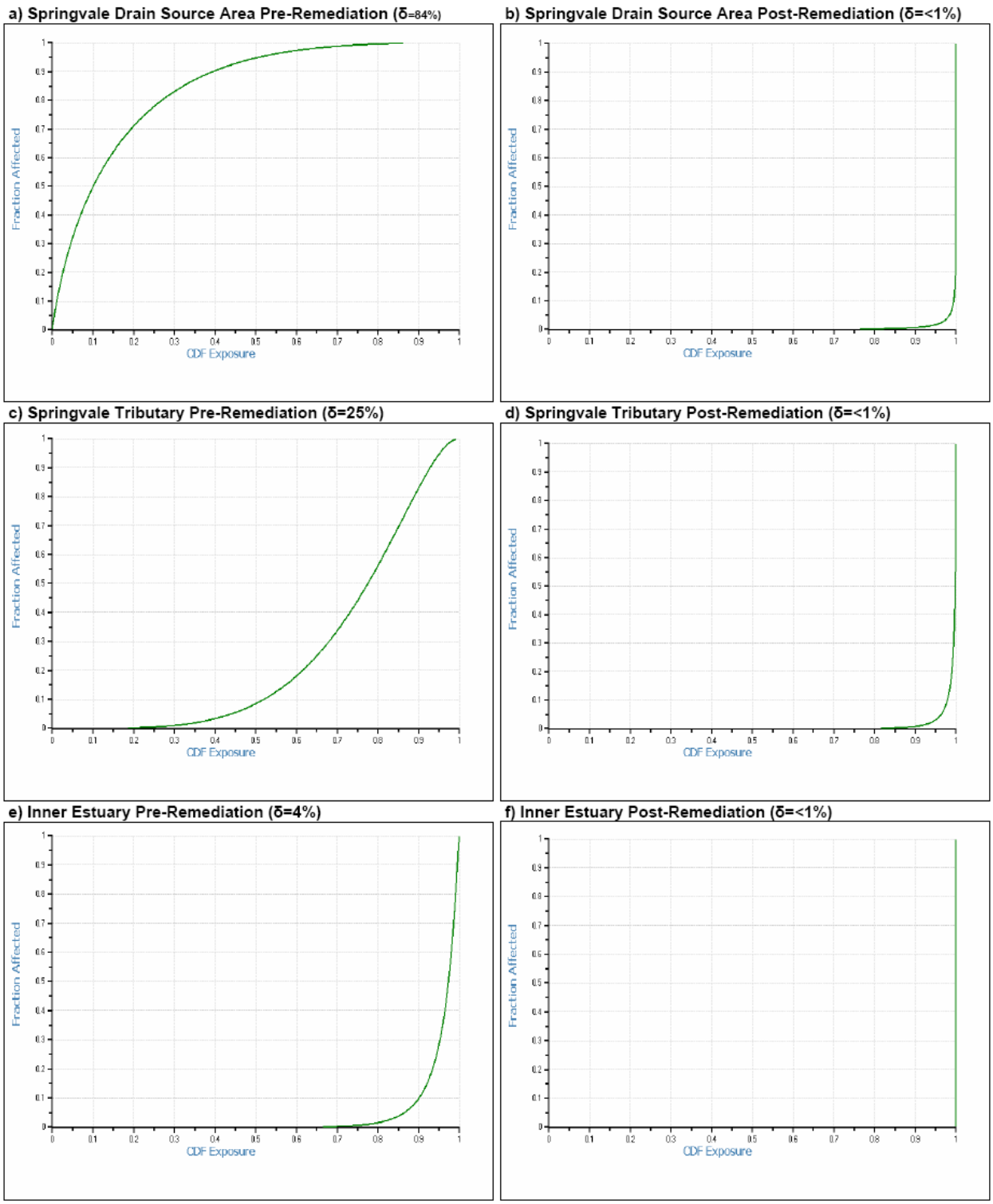


Figure 2a) to 2f). Joint probability curves (JPCs) for ecological risk for pre-remediation and post-remediation scenarios in each of Springvale Drain source area (a, b), in Springvale Tributary (c, d) and in the inner estuary (e, f) across both tides.

UNCERTAINTY

The purpose of including a discussion of uncertainty in the risk assessment is to inform risk managers and decision makers of the uncertainty that exists with the information presented. As was the case for the risk assessment undertaken prior to the remediation, the treatment of uncertainty presented here only identifies sources of uncertainty and does not convey the potential extent or impact of the uncertainty associated with the risk assessment, it is nonetheless important to be explicit with all the sources of uncertainty to ensure that the risk assessment is transparent (Calow 1998). Sources of uncertainty, discussed in detail in Hunt et al. (*in press a*), included: toxicity testing undertaken at constant exposure concentrations; the use of SSDs with the inherent assumption that protection of a proportion of species will protect ecosystem structure and function; and the application of log-normal distributions to right-skewed exposure data.

CONCLUSIONS

The present study demonstrated the use of the joint probability curve (JPC) technique for site-specific ERA to quantify the reduction in ecological risk posed by VCH contamination in Penrhyn Estuary following commissioning of a groundwater remediation system.

The site-specific nature of the toxicity and exposure distributions greatly increase the relevance of the risk assessment. Assessment of the risk following remediation was essential in quantifying the reduction in risk, which was not possible with measurement of concentrations of VCHs alone. Monitoring changes and the implications on ecological risk is vital to the success of the ERA and the risk management of the estuary.

Both JPCs and risk values (δ) indicate that the groundwater remediation has had a strong positive impact on the conditions in the estuary and that the reduction in risk from VCH contamination has decreased to acceptably low levels, where recovery of the aquatic ecosystem would be expected.

CONCLUSIONS

Previous investigations identified groundwater contaminated with volatile chlorinated hydrocarbons (VCHs) at a chemical manufacturing facility in Botany, Sydney. Additional studies identified contamination of surface water with a complex mixture of 14 VCHs in nearby Penrhyn Estuary, a small intertidal embayment on the northern margin of Botany Bay (Woodward-Clyde 1996). In the current study, a screening level hazard assessment of the contamination was undertaken using the hazard quotient (HQ) approach. Low reliability trigger values (TVs) were derived for 5 VCHs: 1,1,1,2-tetrachoroethane, 1,1-dichloroethane, 1,1-dichloroethene, *cis* 1,2-dichloroethene and *trans* 1,2-dichloroethene, for which water quality guidelines were not previously available. The new TVs ranged from 380 µg/L to 3900 µg/L and were used with the existing TVs for VCHs to assess the hazard posed by VCH contamination of Penrhyn Estuary. The assessment indicated that the hazard was always greater at low tide than at high tide and the VCHs which posed the greatest hazard were 1,2-dichloroethane, tetrachloroethene and vinyl chloride. A high hazard (HQ>1) was identified at 6 out of 9 sites, for at least one contaminant. The potential toxicity of the mixture was greater than for individual contaminants, however, the number of sites where there was a high hazard did not increase. The screening level hazard assessment also identified several limitations including: the low reliability of the TVs for VCHs provided in ANZECC and ARMCANZ (2000); the limited applicability of the TVs to a complex mixture of 14 potentially interacting contaminants and the need to undertake direct toxicity assessment (DTA) of the mixture; the use of deterministic measures for exposure and toxicity profiles in the hazard quotient method, which do not account for spatial and temporal variability in VCH concentrations; and the lack of any elements of probability to assess 'risk'. Subsequent studies were undertaken to address the identified shortcomings of the screening level hazard assessment.

Due to the volatile nature of VCHs, these chemicals would be quickly lost from the open test vessels used routinely in toxicity testing. Therefore, a toxicity testing methodology using sealed test vessels was developed and evaluated for its suitability in preventing loss of VCHs from test solutions and also for testing with 6 indigenous marine organisms

including; oyster (*Saccostrea commercialis*) and sea urchin larvae (*Heliocidaris tuberculata*); a benthic alga (*Nitzschia closterium*); an amphipod (*Allorchestes compressa*); a larval fish (*Macquaria novemaculeata*); and a polychaete worm (*Diopatra dentata*). Of the test organisms, larval fish (*M.novemaculeata*) had only been used in 2 other published studies (Cohen and Nugegoda 2000; Cohen *et al.* 2003) and the polychaete (*D.dentata*) had not previously been used. The methodology was evaluated with 3 experimental treatments, including: a complex mixture of VCHs in groundwater; and seawater spiked individually with 1,2-dichloroethane and chloroform. Results indicated that the vials used for small organisms were effective in preventing losses of VCHs; however, on average, 46% VCH loss occurred in jars used for testing medium-sized organisms. The greater loss in jars was attributed to the presence of approximately 50% headspace left to allow for the greater oxygen demand of the medium-sized organisms. Survival and precent normal development in the artificial salt water (ASW) controls for the amphipod (84%) and oyster (69%) tests were marginally below accepted criteria of 90% and 70%, respectively, indicating that the artificial salt may be marginal for these test organisms. For the larval fish test, survival (53%) was below the criteria of 80% for the filtered seawater control (FSW). The survival in the lowest exposure treatments for 1,2-dichloroethane and chloroform exceeded the acceptance criteria, indicating that the survival in the test vessel was acceptable, however, further development of the test protocol may be required. Water quality parameters in the test vessels, i.e. dissolved oxygen content and pH, were maintained throughout the duration of the testing. The assessment concluded that the test containers were generally suitable in preventing loss of VCHs and were acceptable for use with the test organisms.

Following identification in the screening level hazard assessment of the complex mixture of VCHs and requirement to undertake DTA, testing was undertaken using groundwater contaminated with VCHs using the 6 indigenous marine organisms above. No observed effect concentration (NOEC) values varied from 1.56% dilution (1.11 mg total VCHs) to 50 % dilution (45.5 mg total VCHs). EC50 values varied from 4.8% dilution (3.77 mg total VCHs) to >50% dilution (45.5 mg total VCHs). NOEC data were used to derive species sensitivity distributions (SSD) and a site-specific guideline. SSDs were derived

using Burr Type III (including the Pareto) and log-normal distributions. The log-normal distribution represented the best fit and as the Pareto distribution is a finite threshold model more suited to toxicants with a threshold mode of action, the log-normal SSD and the associated 95% trigger value (TV) of 830 µg/L of total VCHs, was adopted as the site-specific TV for the groundwater. The SSD was evaluated using an acute to chronic ratio (ACR) of 5 and treating the 2 sub-chronic larval development tests as chronic tests (i.e. no ACR applied). The effect of the choice of ACR of either 5 or 10 and inclusion of sub-chronic tests as either acute or chronic varied the TVs by up to threefold. The TV derived in the current study was similar to the predicted TV of 1 800 µg/L when the complex mixture was evaluated using the toxic unit (TU) approach. The small number of indigenous species available for toxicity testing and the even smaller number of species for which chronic tests are available, greatly affected the choice of tests and possibly, the derived distributions and guideline values. Therefore, continued development of chronic indigenous test organisms is recommended. However, the current study demonstrated that a site-specific, risk-based guideline for a complex mixture of VCHs may be derived using an SSD attained from DTA on a battery of indigenous test species.

A higher tier, probabilistic ecological risk assessment was undertaken to address the identified limitations of the hazard assessment. The risk assessment incorporated probabilistic elements for toxicity and exposure and used the joint probability curve (JPC) methodology to derive quantitative estimates of ecological risk (δ) and an understanding of the type of exposure of aquatic organisms in the receiving environment. The ERA used the SSD derived in the DTA as the toxicity assessment and monitoring from surface water contamination at the site in the exposure assessment. Risk was characterised in the source areas in Springvale Drain and Floodvale Drain and in each of the areas within the estuary – Springvale and Floodvale Tributaries and the inner and outer estuary. Risk was characterised at high- and low tides individually and when data from both high- and low tides were assessed together. The risk of possible adverse effects and likely adverse effects were assessed with SSDs derived using NOEC and EC50 data, respectively. Estimates of risk varied from a maximum of 84% in the source areas, to 35% in the Springvale Tributary and <1% in the outer estuary. Significant risks (i.e. >5%) in the NOEC scenario

were identified in the estuary in the Springvale and Floodvale Tributaries and in the inner estuary. In the EC50 scenario, significant risks in the estuary were restricted to Springvale Tributary. Risk was greatest at low tide followed by both high- and low tides assessed together, with the lowest risk being at high tide. The 2 toxicity scenarios also allowed investigation of different levels of risk, i.e. for protection of organisms and possible ecological effects (NOEC) or assessment of significant adverse ecological effects (EC50). The shape of the curve and magnitude of the risk each support the generation of ecological hypotheses on the type of exposure and the ecological community likely to be present, for future evaluation. This ERA demonstrated a 'best practice', probabilistic ERA using site-specific probability distributions for exposure and toxicity assessments. Ecological risk was quantified by estimation of the extent of overlap of the toxicity and exposure distributions. The site-specific nature of the toxicity and exposure distributions greatly increase the relevance of the risk assessment.

VCHs in the current study act under the narcotic pathway, inhibiting cellular processes through interference with membrane integrity and are additive in toxicity. Lethal toxicity (i.e. LC50) is typically reported at the internal lethal concentration to 50% or organisms (ILC50) or critical body residue (CBR) of ~2.5 mmol/kg wet weight. The objectives of this study were to assess the sensitivity of indigenous species and to evaluate if additivity of VCHs in groundwater accounted for observed toxicity. Predicted internal residues for 5 test organisms were derived for the mixture of VCHs in groundwater and seawater spiked individually with chloroform and 1,2-dichloroethane using bioconcentration factors. Predicted residues (at LC50/EC50) were typically between 1 and 10 mmol/kg, with the exception of the algal and sea urchin toxicity tests, which were considerably lower than the expected minimum by up to 2 orders of magnitude (sea urchin). Mean internal residues for the groundwater, chloroform and 1,2-dichloroethane were 0.88 mmol/kg, 2.84 mmol/kg and 2.32 mmol/kg, respectively, i.e. close to the predicted value of ~2.5 mmol/kg, indicating that the organisms were suitably sensitive to VCHs. The low exposure concentrations at which effects were observed in the algal and sea urchin tests could be indicative of effects to sensitive endpoints (i.e. growth and development) at lower concentrations, rather than effects at the relatively high narcotic threshold, similar to the

findings of other studies. The ILC50 predicted from the individual components of the contaminated groundwater was assessed to be not significantly different from the ILC50s from individual spike tests and also within the expected range for narcotic contaminants. The additive toxicity of VCHs in groundwater was, therefore, considered to account for the toxicity observed in the DTA. Assessment of predicted ILC50s based on exposure concentrations and bioconcentration factors provided a suitable, cost-effective method to evaluate the potential toxicity of a contaminant mixture, without the need to undertake additional toxicity testing or evaluation.

Toxicity testing was undertaken to evaluate whether the existing low reliability ANZECC and ARMCANZ (2000) TVs for chloroform and 1,2-dichloroethane are protective of indigenous marine organisms. No observed effect concentrations (NOECs) for 1,2-dichloroethane varied from 580 to 159 000 µg/L and for chloroform, the NOECs varied from 4 µg/L to 55 200 µg/L. EC50s for 1,2-dichloroethane varied from 17 500 µg/L to 245 000 µg/L and for chloroform, the EC50s varied from 122 µg/L to 98 800 µg/L. The TVs were protective of 4 of the 6 species tested, (*A.compressa*, *D.dentata*, *S.commercialis* and *M.novemaculeata*), however, the TVs were not protective of the alga (*N.closterium*) or the sea urchin larvae (*H.tuberculata*), with NOECs considerably less than existing TVs. As the existing TVs were not considered to be adequately protective, SSDs and new TVs were derived using NOEC data generated from the testing in accordance with the methodology outlined in ANZECC and ARMCANZ (2000). New, moderate reliability 95% TVs were derived for 1,2-dichloroethane (165 µg/L) and for chloroform (2 µg/L). The Trigger Values derived were between 1 and 3 orders of magnitude lower than the existing ANZECC and ARMCANZ (2000) low reliability TVs. The derivation of TVs in the current study may be dependent on the small number of test organisms and the selection of sensitive test endpoints used in the derivation. The revision of data for volatile contaminants when toxicity testing was undertaken in sealed containers was, however, consistent with the findings of other researchers and suggests the need to evaluate the protectiveness of the TVs derived for other VCHs. Further testing of volatile chlorinated hydrocarbons is needed to support both the current study and the existing guidelines. Further development of indigenous chronic bioassays is urgently required.

Following identification of potential ecological risks to aquatic receptors resulting from groundwater contamination in Penrhyn Estuary, a groundwater remediation system was commissioned in 2006 to prevent the discharge of groundwater containing VCHs into Penrhyn Estuary and Botany Bay. The success of the project had, however, only been measured in engineering or chemical measures. As the ultimate objective of the remediation was to reduce the potential ecological risks to aquatic receptors and the ecosystem, it was more appropriate to evaluate the success of the project with regard to changes in ecological risk. To assess the ecological risk following implementation of the groundwater treatment system, a risk assessment was undertaken using the toxicity data derived from the DTA and surface water monitoring data collected during 2007 and 2008. The assessment indicated that, ecological risk reduced in the source areas from a maximum of 84% prior to remediation, to a maximum of only 1.4%, after remediation. In the estuary, risk decreased from a maximum of 35% to only ~1% after remediation and that risk in all areas of the estuary was acceptable (i.e. <5%). The present study demonstrated the use of the JPC technique for site-specific ERA to successfully quantify the reduction in ecological risk of VCH contamination in Penrhyn Estuary following commissioning of a groundwater remediation system. JPCs and risk values (δ) both indicated that groundwater remediation had had a strong positive impact on conditions in the estuary and that the reduction in risk from VCH contamination had decreased to acceptably low levels, where recovery of the aquatic ecosystem would be expected. The site-specific nature of the toxicity and exposure distributions greatly increased the relevance of the risk assessment and revisiting the risk assessment following a change in conditions 'completed the loop' in the risk management cycle for the estuary.

The current study presented a 'best-practice', quantitative, probabilistic ERA of groundwater contaminated with a complex mixture of VCHs being discharged into an adjacent estuarine embayment. The ERAs presented utilised site-specific measures for both toxicity and exposure assessments. Toxicity of the VCHs in groundwater and the sensitivity of the indigenous test organisms used in the risk assessment were evaluated using internal critical residues of VCHs. Evaluation of the existing low reliability

ANZECC and ARMCANZ (2000) TVs for VCHs was undertaken and new, moderate reliability TVs were derived for 2 VCHs. Revision of the ecological risk assessment was undertaken to quantify the reduction in ecological risk in Penrhyn Estuary following commissioning of the groundwater remediation system. Revision of the risk assessment as conditions change is crucial to the success of the ecological risk management framework.

REFERENCES

Abernathy, S.G., Mackay, D., and McCarty, L.S. 1988. "Volume Fraction" correlation for narcosis in aquatic organisms: the key role of partitioning. *Environmental Toxicology & Chemistry* 7, 469-481.

AGEE, and Woodward-Clyde. 1990. Stage 1 Preliminary Investigation, ICI Botany Environmental Survey, AG Environmental Engineers & Woodward Clyde Consultants Inc, May 1990.

Aldenberg, T., Jaworska, J., and Trass, T.P. 2002. Normal Species Sensitivity Distributions and Probabilistic Ecological Risk Assessment. In Posthuma, L., Suter, G.W., and Trass, T.P., eds., *Species Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, Florida.

Altenburger, R., Backhaus, T., Boedeker, W., Faust, M., Scholze, M., and Grimme, L.H. 2000. Predictability of the toxicity of multiple chemical mixtures to *Vibrio fischeri*: Mixtures composed of similarly acting chemicals. *Environmental Toxicology and Chemistry* 19, 2341-2347.

ANZECC and ARMCANZ. 2000. *Australian and New Zealand Guidelines for Fresh and Marine Water Quality.* Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, National Water Quality Management Strategy.

APHA. 1998. *Standard Methods for the Examination of Water and Wastewater. 20th Ed.* American Public Health Association, American Water Works Association and the Water Environment Federation, Washington, DC.

ASTM. 1995. *Standard guide for conducting static acute toxicity tests with echinoid embryos. ASTM E-1563, Annual Book of ASTM Standards, Volume 11.04*. American Society for Testing and Materials, Philadelphia.

AWT ES&T, P.L. 1996. *Toxicity testing of sewage effluent from coastal STPs in the Sydney and Illawarra regions.* Sydney Water Corporation.

Bartell, S.M. 1996. Some thoughts concerning quotients, risks and decision making. *Human and Ecological Risk Assessment*.

Bervoets, L., Baillieul, M., Blust, R., and Verheyen, R. 1996. Evaluation of effluent toxicity and ambient toxicity in a polluted lowland river. *Environmental Pollution* 91, 333-341.

Bickford, G., Toll, J., Hansen, J., Baker, E., and Keessen, R. 1999. Aquatic ecological and human health risk assessment of chemicals in wet weather discharges in the Sydney region, New South Wales, Australia. *Marine Pollution Bulletin* 39, 335-345.

Birch, G., Evanden, D., and Teutch, M.E. 1996. Dominance of point sources in heavy metal distributions in sediment of a major Sydney estuary (Australia). *Environmental Geology* 28, 169-174.

Birch, G., and Taylor, S. 1999. Source of heavy metals in sediments of the Port Jackson estuary, Australia. *Science of the Total Environment* 227, 123-138.

Bossuyt, B.T.A., Muyssen, B.T.A., and Janssen, C.R. 2005. Relevance of generic and site-specific species sensitivity distributions in the current risk assessment procedures for copper and zinc. *Environmental Toxicology and Chemistry* 24, 470-478.

Brack, W., Rottler, H., and Frank, H. 1998. Volatile fractions of landfill leachates and their effect on *Chlamydomonas reinhardtii*: In vivo chlorophyll a fluorescence. *Environmental Toxicology and Chemistry* 17, 1982-1991.

Bradbury, S.P., and Lipnick, R.L. 1990. Symposium on structural properties for determining mechanisms of toxic action, Duluth MI, USA, October 18–20 1988. *Environmental Health Perspectives* 87, 181.

Bradbury, S.P., Russom, C.L., Ankley, G.T., Schultz, T.W., and Walker, J.D. 2003. Overview of data and conceptual approaches for derivation of quantitative structure-activity relationships for ecotoxicological effects of organic chemicals. *Environmental Toxicology and Chemistry* 22, 1789-1798.

Brix, K.V., DeForest, D.K., and Adams, W.J. 2001. Assessing acute and chronic copper risks to freshwater aquatic life using species sensitivity distributions for different taxonomic groups. *Environmental Toxicology and Chemistry* 20, 1846-1856.

Broderius, S.J., Kahl, M.D., Elonen, G.E., Hammermeister, D.E., and Hoglund, M.D. 2005. A comparison of the lethal and sublethal toxicity of organic chemical mixtures to the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry* 24, 3117-3127.

Broderius, S.J. and Kahl, M.D. 1985. Acute toxicity of organic chemical mixtures to the fathead minnow. *Aquatic Toxicology* 6, 307-322.

Brown, P.L., and Ferris, J.M. 2004. Risk-Based Assessment of the Impact of Aluminium on a Riverine Ecosystem. *Australian Journal of Chemistry* 57, 951-955.

Burgman, M. 2005. *Risks and Decisions for Conservation and Environmental Management*. Cambridge University Press, Cambridge.

Burton, D.T., Dilorenzo, J.L., Shedd, T.R., and Wrobel, J.G. 2002. Aquatic Hazard Assessment of a Contaminated Surficial Aquifer Discharge into the Bush River, Maryland (USA). *Water Air and Soil Pollution* 139, 159-182.

Calabrese, E., and Baldwin, L. 1993. *Performing Ecological Risk Assessments*. Lewis Publishers, Boca Raton, Florida, USA.

Calow, P. 1998. Ecological risk assessment: Risk for what? How do we decide? *Ecotoxicology and Environmental Safety* 40, 15-18.

Campbell, E., Palmer, M.J., Shao, Q., Warne, M., StJ., and Wilson, D. 2000. *BurrliOZ: A computer program for calculating toxicant trigger values for the ANZECC and ARMCANZ Water Quality Guidelines*. Perth, W.A.

Campbell, P.J., Arnold, D.J.S., and Brock, T.C.M. 1999. *Guidance document on Higher Tier Aquatic Risk Assessment for Pesticides (HARAP).* SETAC Europe, Brussels, Belgium.

Capdevielle, M., van Egmond, R., Whelan, M., Versteeg, D., Hofmann-Kamensky, M., Inauen, J., Cunningham, V., and Woltering, D. 2008. Consideration of exposure and

species sensitivity of Triclosan in the freshwater environment. *Integrated Environmental Assessment and Management* 4, 15-23.

Carey, J., Cook, P., Giesy, J., Hodson, P., Muir, D., Owens, W., and Soloman, K. 1998. *Ecotoxicological Risk Assessment of the Chlorinated Organic Chemicals*. Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, Florida.

Chapman, J. 2001. The Revised Australian and New Zealand Water Quality Guidelines for Toxicants: Approach to their Derivation and Application. *Australasian Journal of Ecotoxicology* 7, 95-108.

Chapman, P.M., Fairbrother, A., and Brown, D. 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. *Environmental Toxicology & Chemistry* 17, 99-108.

Chen, C.-Y., and Lin, J.-H. 2005. Toxicity of chlorophenols to *Pseudokirchneriella subcapitata* under air-tight test environment. *Chemosphere* 62, 503-509.

Cohen, A., Gagnon, M.M., and Nugegoda, D. 2003. Biliary PAH metabolite elimination in Australian bass, *Macquaria novemaculeata* following exposure to bass strait crude oil and chemically dispersed crude oil. *Bulletin of Environmental Contamination and Toxicology* 70, 394-400.

Cohen, A., and Nugegoda, D. 2000. Toxicity of Three Oil Spill Remediation Techniques to the Australian Bass *Macquaria novemaculeata*. *Ecotoxicology and Environmental Safety* 47, 178-185.

Connell, D.W., Chaisuksant, Y., and Yu, J. 1999. Importance of internal biotic concentrations in risk evaluations with aquatic systems. *Marine Pollution Bulletin* 39, 54-61.

Cunningham, V.L., Constable, D.J.C., and Hannah, R.E. 2004. Environmental risk assessment of paroxetine. *Environmental Science & Technology* 38, 3351-3359.

Daniels, R.E., and Allen, J.D. 1981. Life table evaluation of chronic exposure to a pesticide. *Canadian Journal of Fisheries and Aquatic Science* 38, 485-494.

Day, K.E., and Kaushik, N.K. 1987. An assessment of the chronic toxicity of the synthetic pyrethroid, fenvalerate, to *Daphnia galeata mendotae*, using life tables. *Environmental Pollution* 44.

De Rooij, C., Boutonnet, J.-C., Garny, V., Lecloux, A., Papp, R., Thompson, R.S., and van Wijk, D. 1998. Euro chlor risk assessment for the marine environment Osparcom region: North Sea - 1,2-dichloroethane. *Environmental Monitoring and Assessment* 53, 425-445.

De Zwart, D. 2002. Observed regularities in species-sensitivity distributions for aquatic species, 133-154. In Posthuma, L., Suter, G.W., and Traas, T.P., eds., *Species Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Ration, Florida.

Di Toro, D.M., and McGrath, J.A. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. *Environmental Toxicology and Chemistry* 19, 1971-1982.

Di Toro, D.M., McGrath, J.A., and Hansen, D.J. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. *Environmental Toxicology and Chemistry* 19, 1951-1970.

Diamond, J., and Daley, C. 2000. What is the relationship between whole effluent toxicity and instream biological condition? *Environmental Toxicology and Chemistry* 19, 158-168.

Dinnel, P.A., Link, J.M., and Stober, Q.J. 1987. Improved methodology for a sea urchin sperm cell bioassay for marine waters. *Archives of Environmental Contamination & Toxicology* 16, 23-32.

Doyle, C.J., Pablo, F., Lim, R.P., and Hyne, R.V. 2003. Assessment of metal toxicity in sediment pore water from Lake Macquarie, Australia. *Archives of Environmental Contamination and Toxicology* 44, 343-350.

Duboudin, C., Ciffroy, P., and Magaud, H. 2004. Effects of data manipulation and statistical methods on species sensitivity distributions. *Environmental Toxicology and Chemistry* 23, 489-499.

Dyer, S., White-Hull, C., and Shephard, B. 2000. Assessments of chemical mixtures via toxicity reference values overpredict hazard to Ohio fish communities. *Environmental Science and Technology* 34, 2518-2524.

ECOFRAM. 1999. ECOFRAM Terrestrial Draft Report. Ecological Committee on FIFRA Risk Assessment Methods (ECOFRAM), May 1999.

Edgar, G.J. 1997. *Australian Marine Life*. Reed Books, Kew, Victoria.

Escher, B.I., and Hermens, J.L.M. 2002. Modes of action in ecotoxicology: Their role in body burdens, species sensitivity, QSARs, and mixture effects. *Environmental Science and Technology* 36, 4201-4217.

Escher B, Ashauer R, Dyer S, Hermens J, Lee JH, Mayer P, Leslie H, and Warne MStJ. *in press*. The crucial role of toxic modes and mechanisms of action for tissue residues. In *The Tissue Residue Approach in Ecotoxicology.*

Forbes, T.L., and Forbes, V.E. 1993. A critique of the use of distribution-based extrapolation models in ecotoxicology. *Functional Ecology* 7, 249-254.

Galassi, S., and Vighi, M. 1981. Testing toxicity of volatile substances with algae. *Chemosphere* 10, 1123-1126.

Garny, V., Lecloux, A., and Papp, R. 1998. Euro chlor risk assessment for the marine environment osparcom region: North Sea - principles and purposes. *Environmental Monitoring and Assessment* 53, 395-399.

Giddings, J.M., Solomon, K., and Maund, S.J. 2001. Probabilistic risk assessment of cotton pyrethroids: II Aquatic mesocosm and field studies. *Environmental Toxicology & Chemistry* 20, 660-668.

Gilbert, R.O. 1987. *Statistical Methods for Environmental Pollution Monitoring*. van Nostrand Reinhold, New York.

Grothe, D.R., Dickson, K.L., and Reed-Judkins, D.K. 1996. *Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts*. Society of Environmental Toxicology and Chemistry (SETAC) Press, Pensacola, Florida.

Hall, L.W., and Giddings, J.M. 2000. The need for multiple lines of evidence for predicting site-specific ecological effects. *Human and Ecological Risk Assessment* 6, 679-710.

Hall, L.W., Scott, M.C., and Killen, W.D. 1998. Ecological risk assessment of copper and cadmium in surface waters of Chesapeake Bay watershed. *Environmental Toxicology and Chemistry* 17, 1172-1189.

Hanson, M.L., and Solomon, K.R. 2002. New technique for estimating thresholds of toxicity in ecological risk assessment. *Environmental Science and Technology* 36, 3257-3264.

Hart, B.T., Burgman, M., Grace, M.R., Pollino, C.A., Thomas, C., and Webb, J.A. 2006. Risk Based Approaches to Managing Contaminants in Catchments. *Human and Ecological Risk Assessment* 12, 66-73.

Hart, B.T., Grace, M.R., Breen, P., Cottingham, P., Feehan, P., and Burgman, M. 2001. Application of ecological risk assessment in river management, 289-295. In Rutherford, I., Sheldon, F., Brierly, G., and Kenyon, C., eds., *Proceedings of the Third Australian Stream Management Conference - Value of Healthy Streams.* CRC for Catchment Hydrology, Melbourne.

Hart, B.T., Lake, P.S., Webb, J.A., and Grace, M.R. 2003. Ecological risk to aquatic systems from salinity increases. *Australian Journal of Botany* 51, 689-702.

Hela, D.G., Lambropoulou, D.A., Konstantinou, A.K., and Albanis, T.A. 2005. Environmental monitoring and ecological risk assessment for pesticide contamination and effects in Lake Pamvotis, northwestern Greece. *Environmental Toxicology and Chemistry* 24, 1548-1556.

Herman, D.C., Inniss, W.E., and Mayfield, C.I. 1990. Impact of volatile aromatic hydrocarbons, alone and in combination, on growth of freshwater algae *Selenastrum capricornutum*. *Aquatic Toxicology* 18, 87-100.

Hobbs, D. 2006. Comparison of the sensitivity of Australian and non-Australian aquatic organisms to selected metals, 137. *MSc (Science)*. University of Technology, Sydney, Sydney.

Hogan, A., Stauber, J., Pablo, F., Adams, M., and Lim, R. 2005. The development of marine toxicity identification evaluation (TIE) procedures using the unicellular alga *Nitzschia closterium*. *Archives of Environmental Contamination and Toxicology* 48, 433-443.

Hose, G., and van den Brink, P. 2004. Confirming the species-sensitivity distribution concept for endosulfan using laboratory, mesocosm, and field data. *Archives of Environmental Contamination and Toxicology* 47, 511-520.

Hose, G.C. 2005. Assessing the Need for Groundwater Quality Guidelines for Pesticides Using the Species Sensitivity Distribution Approach. *Human and Ecological Risk Assessment* 11, 951-966.

Hunt, J.E., Birch, G., and Warne, M., StJ. 2007. Deriving Trigger Values for, and assessing hazard posed by, volatile chlorinated hydrocarbons in a Sydney estuary. *Australasian Journal of Ecotoxicology* 13, 33-42.

Hunt, J.E., Birch, G., Warne, M., StJ., and Krassoi, R. 2009a. Evaluation of a methodology for toxicity testing of volatile chlorinated hydrocarbons on marine organisms. *Bulletin of Environmental Contamination and Toxicology* 82, 743-748.

Hunt, J.E., Birch, G., Warne, M., StJ., and Krassoi, R. 2009b. Direct toxicity assessment of volatile chlorinated hydrocarbon contaminated groundwater and derivation of a site-specific guideline. *Integrated Environmental Assessment and Management* 5, 338-348.

Hunt, J.E., Birch, G., and Warne, M., StJ. *in press a*. Quantifying ecological risk assessment of volatile chlorinated hydrocarbon contamination in a tidal estuary.

Hunt, J.E. *in press b*. Using a critical body residue approach to evaluate species sensitivity and the additive toxicity of volatile chlorinated hydrocarbons in groundwater.

Jin, H., Yang, X., Yin, D., and Yu, H. 1999. A case study on identifying the toxicant in effluent discharged from a chemical plant. *Marine Pollution Bulletin* 39, 122-125.

Johnson, I., Hutchings, M., Benstead, R., Thain, J., and Whitehouse, P. 2004. Bioassay selection, experimental design and quality control/assurance for use in effluent assessment and control. *Ecotoxicology* 13, 437-447.

Jones, E.J.P., Voytek, M.A., and Lorah, M.M. 2004. Effect of Fe(III) on 1,1,2,2-tetrachloroethane degradation and vinyl chloride accumulation in wetland sediments of the Aberdeen Proving Ground. *Bioremediation Journal* 8, 31-45.

Kefford, B.J., Palmer, C.G., Jooste, S., Warne, M., StJ., and Nugegoda, D. 2005. What is meant by "95% of species"? An argument for the Inclusion of Rapid Tolerance Testing. *Human and Ecological Risk Assessment* 11, 1025-1046.

Krassoi, R. 1996. Adaptation of the 48 h larval abnormality test using the doughboy scallop and Sydney rock oyster to the assessment of sediments, O108. *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Sydney.

Kszoz, L.A., Talmage, S.S., Konetsky, B.K., and Rottero, T. 2003. Derivation of Aquatic Screening Benchmarks for 1,2-Dibromoethane. *Archives of Environmental Contamination & Toxicology* 45, 66-71.

Kwok, K., Bjorgesaeter, A., Leung, K., Lui, G.C.S., Gray, J.S., Shin, P., and Lam, P.K.S. 2008. Deriving site-specific sediment quality guidlines for Hong Kong marine environments using field-based species sensitivity distributions. *Environmental Toxicology & Chemistry* 27, 226-234.

Lakatos, G., Fleit, E., and Meszaros, I. 2003. Ecotoxicological studies and risk assessment on the cyanide contamination of the Tisza river. *Toxicology Letters* 140-141, 333-342.

Lampron, K.J., Chiu, P.C., and Cha, D.K. 2001. Reductive Dehalogenation of Chlorinated Ethenes with Elemental Iron: The Role of Microorganisms. *Water Research* 35, 3077-3084.

Landrum, P.F., Eadie, B.J., and Faust, W.R. 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia sp*. *Environmental Toxicology and Chemistry* 10, 35-46.

Leung, K., Morritt, D., Wheeler, J., Whitehouse, P., Sorokin, N., Toy, R., Holt, M., and Crane, M. 2001. Can saltwater toxicity be predicted from freshwater data? *Marine Pollution Bulletin* 42, 1007-1013.

Lin, J.-H., Kao, W.-C., Tsai, K.-P., and Chen, C.-Y. 2005. A novel algal toxicity testing technique for assessing the toxicity of both metallic and organic toxicants. *Water Research* 39, 1869-1877.

Lotufo, G.R. 1998. Lethal and sublethal toxicity of sediment-associated fluoranthene to benthic copepods: application of the critical-body-residue approach. *Aquatic Toxicology (Amsterdam)* 44, 17-30.

Maltby, L., Blake, N., Brock, T.C.M., and van den Brink, P.J. 2003. *Addressing interspecific variation in sensitivity and the potential to reduce this source of uncertainty in ecotoxicological assessments.* Science and Research Report PN0932. In Department of Environment, F.a.R.A.D., ed. DEFRA, London, UK.

Maltby, L., Blake, N., Brock, T.C.M., and van den Brink, P.J. 2005. Insecticide species sensitivity distributions: Importance of test species selection and relevance to aquatic ecosystems. *Environmental Toxicology and Chemistry* 24, 379-388.

Mayer, P., Nyholm, N., Verbruggen, E.M.J., Hermens, J.L.M., and Tolls, J. 2000. Algal growth inhibition test in filled, closed bottles for volatile and sorptive materials. *Environmental Toxicology and Chemistry* 19, 2551-2556.

McCarty, L.S. 1986. The relationship between aquatic toxicity, QSARs and bioconcentration for some organic chemicals. *Environmental Toxicology and Chemistry* 5, 1071-1780.

McCarty, L.S., and Mackay, D. 1993a. Enhancing Toxicological Modelling and Assessment, Body Residues and Modes of Action. *Environmental Science & Technology* 27, 1719-1728.

McCarty, L.S., and Mackay, D. 1993b. Enhancing Toxicological Modelling and Assessment, Body Residues and Modes of Action. *Environmental Science & Technology* 27, 1719-1728.

McGrath, J.A., Parkerton, T.F., and Di Toro, D.M. 2004. Application of the narcosis target lipid model to algal toxicity and deriving predicted-no-effect concentrations. *Environmental Toxicology and Chemistry* 23, 2503-2517.

Meador, J. 2006. Rational and procedures for using the tissue-residue approach for toxicity assessment and determination of tissue, water and sediment quality guidelines for aquatic organisms. *Human and Ecological Risk Assessment* 12, 1018-1073.

Muschal, M., and Warne, M., StJ. 2003. Risk Posed by Pesticides to Aquatic Organisms in Rivers of Northern Inland NSW, Australia. *Human and Ecological Risk Assessment* 9, 1765-1787.

NEPC. 1999. Schedule B(5) *Guideline on Ecological Risk Assessment. National Environment Protection Measure (Assessment of Site Contamination).* Ed. National Environment Protection Council, 45 pp.

Newman, M.C., Ownby, D.R., Mezin, L.C.A., Powell, D.C., Christensen, T.R.L., Lerberg, S.B., and Anderson, B.-A. 2000. Applying species-sensitivity distributions in ecological risk assessment: Assumptions of distribution type and sufficient numbers of species. *Environmental Toxicology and Chemistry* 19, 508-515.

Nirmalakhandan, N., Xu, S., Trevizo, C., Brennan, R., and Peace, J. 1997. Additivity in microbial toxicity of nonuniform mixtures of organic chemicals. *Ecotoxicology and Environmental Safety* 37, 97-102.

Pankow, J.F., Asher, W.E., and Zogorski, J.S. 2006. Source Apportionment Modeling of Volatile Organic Compounds in Streams. *Environmental Toxicology & Chemistry* 25, 921-932.

Plackett, R., and Hewlett, P. 1952. Quantal responses to mixtures of poisons. *Journal of the Royal Statistical Society B* 14, 141.

Pohl, H.R., Roney, N., Wilbur, S., Hansen, H., and De Rosa, C.T. 2003. Six interaction profiles for simple mixtures. *Chemosphere* 53, 183-197.

Poletika, N.N., Woodburn, K.B., and Henry, K.S. 2002. An Ecological Risk Assessment for Chlorpyrifos in an Agriculturally Dominated Tributary of the San Joaguin River. *Risk Analysis* 22, 291-308.

Posthuma, L., Suter, G.W., and Trass, T.P. 2002. *Species Sensitivity Distributions in Ecotoxicology*. Lewis Publishers, CRC Press, Boca Raton.

Reiss, R., Mackay, N., Habig, C., and Griffin, J. 2002. An ecological risk assessment for triclosan in lotic systems following discharge from wastewater treatment plants in the United States. *Environmental Toxicology and Chemistry* 21, 2483-2492.

Ren, S. 2002. Predicting three narcosis mechanisms of aquatic toxicity. *Toxicology Letters* 133, 127-139.

Ritter, A.M., Shaw, J.L., Williams, W.M., and Travis, K.Z. 2000. Characterizing aquatic ecological risks from pesticides using a diquat dibromide case study. I. Probabilistic exposure estimates. *Environmental Toxicology & Chemistry. 19(3). March, 2000. 749-759.*

Roberts, S.M. 1999. Practical issues in the use of probabilistic risk assessment *Human and Ecological Risk Assessment* 5, 729-736.

Rose, R., Warne, M.S.J., and RP, L. 1998. Quantitative structure-activity relationships and volume faction analysis for non-polar narcotic chemicals to the Australian cladoceran, *Ceriodaphnia cf dubia*. *Archives of Environmental Contamination and Toxicology* 34, 248-252.

Rose, R.M., Warne, M., StJ., and Lim, R.P. 1997. Inter-species conversion equations for predicting toxicity of non-polar narcotic chemicals to *Ceriodaphnia dubia*. *Australasian Journal of Ecotoxicology* 3, 75-83.

Schmitt-Jansen, M., and Altenburger, R. 2005. Predicting and observing responses of algal communities to photosystem II-herbicide exposure using pollution-induced community

tolerance and species-sensitivity distributions. *Environmental Toxicology and Chemistry* 24, 304-312.

Schultz, T.W. 1989. Nonpolar narcosis: A review of the mechanism of action for baseline toxicity, 104. In Cowgill, U.M., and Williams, L.R., eds., *Aquatic Toxicology and Environmental Fate, vol. 12. ASTM STP 1027*. American Society of Testing and Materials, Philadelphia, PA.

Shao, Q. 1990. Estimation for hazardous concentrations based on NOEC toxicity data: An alternative approach. *Environmetrics* 11, 583-595.

Sijm, D.T.H.M., Schipper, M., and Opperhuizen, A. 1993. Toxicokinetics of halogenated benzenes in fish: lethal body burden as a toxicological endpoint. *Environmental Toxicology and Chemistry* 12, 1117-1127.

Simpson, S.L., Roland, M.G.E., Stauber, J.L., and Batley, G.E. 2003. Effect of declining toxicant concentrations on algal bioassay endpoints. *Environmental Toxicology and Chemistry* 22, 2073-2079.

Solomon, K., Giesy, J., and Jones, P. 2000. Probabilistic risk assessment of agrochemicals in the environment. *Crop Protection* 19, 649-655.

Solomon, K.R. 1996. Overview of recent developments in ecotoxicological risk assessment. *Risk Analysis* 16, 627-633.

Solomon, K.R., Giddings, J.M., and Maund, S.J. 2001. Probabilistic risk assessment of cotton pyrethroids: I. Distributional analysis of laboratory aquatic toxicity data. *Environmental Toxicology & Chemistry* 20, 652-659.

Solomon, K.R., and Sibley, P. 2002. New concepts in ecological risk assessment: Where do we go from here? *Marine Pollution Bulletin* 44, 279-285.

Solomon, K.R., and Takacs, P. 2002. Probabilistic Risk Assessment Using Species Sensitivity Distributions. In Posthuma, L., Suter, G.W., and Trass, T.P., eds., *Species Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton.

Sorenson, M.T., Gala, W.R., and Margolin, J.A. 2004. Approaches to Ecological Risk Characterisation and Management: Selecting the Right Tools for the Job. *Human and Ecological Risk Assessment* 10, 245-269.

Stauber, J. 2003. Sediment Toxicity Testing in Australia. *Workshop on Sediment Quality Assessment*. SETAC/ASE, Christchurch.

Stauber, J.L., Benning, R.J., Hales, L.T., Eriksen, R., and Nowak, B. 2000. Copper bioavailability and amelioration of toxicity in Macquarie Harbour, Tasmania, Australia. *Marine and Freshwater Research* 51, 1-10.

Stauber, J.L., Tsai, J., Vaughan, G., Peterson, S.M., and Brockbank, C.I. 1994. Algae as indicators of toxicity of BEKM effluents. *National Pulp Mills Research Program Technical Report Series No. 3*. CSIRO: Canberra. *pp* 1-82

Stening, J., Fairweather, J., and Lear, J. 2008. Project Contamination Cleanup. *Water Engineering Australia* April 2008, 22-25.

Suter, G.W. 1993. *Ecological Risk Assessment*. Lewis Publishers, Chelsea, Michigan.

Tannenbaum, L.V., Johnson, M.S., and Bazar, M. 2003. Application of the Hazard Quotient Method in Remedial Decisions: A comparison of Human and Ecological Risk Assessments. *Human and Ecological Risk Assessment* 9, 387-401.

The Ecology Lab. 2003. EIS Port Botany Expansion, Aquatic Ecology, Conservation & Fisheries, May 2003, Volume 1: Main Report. The Ecology Lab.

Tinsley, D., Wharfe, J., Campbell, D., Chown, P., Taylor, D., Upton, J., and Taylor, C. 2004. The use of Direct Toxicity Assessment in the assessment and control of complex effluents in the UK: A Demonstration Programme. *Ecotoxicology* 13, 423-436.

Tsai, K.-P., and Chen, C.-Y. 2007. An algal toxicity database of organic toxicants derived by a closed system technique. *Environmental Toxicology & Chemistry* 26, 1931-1939.

Twining, J.R., and Cameron, R.F. 1997. Decision-making processes in ecological risk assessment using copper pollution of Macquarie Harbour from Mt. Lyell, Tasmania, as a case study. *Hydrobiologia* 352, 207-218.

Urban, D.J., and Cook, N.J. 1986. *Hazard Evaluation Division, Standard Evaluation Procedure, Ecological Risk Assessment; EPA540/9-85-001*. U.S. Environmental Protection Agency.

URS. 2004a. *Orica Botany Environmental Survey, Stage 4 – Remediation. 2003 Groundwater and Surface Water Monitoring Report*.

URS. 2004b. *Orica Botany Environmental Survey, Stage 4 – Remediation. 2003 Groundwater and Surface Water Monitoring Report.*

URS. 2005. *Orica Botany Environmental Survey, Stage 4 – Remediation. Variability of Contaminants in Surface Water.*

URS. 2008. *Final Report, Orica Botany Environmental Survey, Stage 4 - Remediation, Groundwater Treatment Plant (GTP) Quarterly Groundwater and Surface Water Monitoring Report, June 2008*.

USEPA. 1990. *Groundwater - Volume 1: Groundwater and Contamination*. In USEPA, ed. Office of Research and Development, Washington DC 20460.

USEPA. 1994. *Short term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Second Edition, EPA/600/4-91/003.* United States Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinatti, OH.

USEPA. 1996a. *Bivalve acute toxicity test (embryo larval) OPPTS 850.1055. Ecological Effects Test Guidelines. EPA/712/C-96/137.* In United States Environmental Protection Agency, P., Pesticides and Toxic Substances, ed.

USEPA. 1996b. *Penaeid acute toxicity test OPPTS 850.1045. Ecological Effects Test Guidelines, EPA/712/C-96/137.* United States Environmental Protection Agency, Prevention, Pesticides and Toxic Substances.

USEPA. 1996c. *Test Methods for Evaluating Solid Waste; Revision 2 Volume B*. U.S. Environmental Protection Agency, Washington D.C.

USEPA. 1998. *Guidelines for Ecological Risk Assessment, EPA/630/R-695/002F*. USEPA, Washington D.C.

USEPA. 2000a. *Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40CFR Part 136).* United States Environmental Protection Agency.

USEPA. 2000b. *Technical Progress Report of the Implementation Plan for Probabilistic Ecological Assessments* - Aquatic Ecosystems. United States Environment Protection Agency.

USEPA. 2002. *Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*. U.S. Environmental Protection Agency.

Vall, M.J.T., van der Wal, J., and Hoekstra, J. 1997. Pattern Analysis in the Sensitivity of Aquatic Species to Toxicants. *Chemosphere* 35, 1291-1309.

van Dam, R.A., Camilleri, C., Bayliss, P., and Markich, S.J. 2004. Ecological risk assessment of Tebuthiuron following application on tropical Australian wetlands. *Human and Ecological Risk Assessment* 10, 1069-1097.

van Dam, R.A., and Chapman, J. 2001. Direct Toxicity Assessment (DTA) for Water Quality Guidelines in Australia and New Zealand. *Australasian Journal of Ecotoxicology* 7, 175-198.

van den Brink, P.J., Brock, T.C.M., and Posthuma, L. 2002. The value of the species sensitivity distribution concept for predicting field effects (non-)confirmation using Semifield experiments., 155-193. In Posthuma, L., Traas, T.P., and Suter, G.W., eds., *Species Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Rqaton, Florida.

van der Hoeven, N. 2001. Estimating the 5-percentile of the species sensitivity distributions without any assumptions about the distribution. *Ecotoxicology* 10, 25-34.

van Hoogen, G.J., and Opperhuizen, A. 1988. Toxicokinetics of chorobenzenes in fish. *Environmental Toxicology and Chemistry* 7, 213-219.

van Leeuwen, C., van der Zandt, P., Aldenberg, T., Verhaar, H., and Hermens, J. 1992. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. 1. Narcotic industial pollutants. *Environmental Toxicology and Chemistry* 11, 267-282.

van Loon, W.M.G.M., Verwoerd, M.E., Wijnker, F.G., van Leeuwen, C.J., van Duyn, P., van Deguchte, C., and Hermens, J.L.M. 1997. Estimating total body residues and baseline toxicity of complex organic mixtures in effluents and surface waters. *Environmental Toxicology and Chemistry* 16, 1358-1365.

van Sprang, P.A., Verdonck, F.A.M., Vanrolleghem, P.A., Vangheluwe, M.L., and Janssen, C.R. 2004. Probabilistic environmental risk assessment of zinc in Dutch surface waters. *Environmental Toxicology and Chemistry* 23, 2993-3002.

van Straalen, N. 2002. Theory of ecological risk assessment based on species sensitivity disbtributions. In Posthuma, L., Suter, G.W., and Traas, T.P., eds., *Species Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, Florida.

van Vlaardingen, P., Trass, T.P., and Aldenberg, T. 2004. ETX 2.0 by RIVM. In van Vlaardingen, P., Trass, T.P., and Aldenberg, T., eds. National Institute of Public Health and Environment. RIVM, Bilthoven, Netherlands, Bilthoven.

van Wezel, A.P., De Vries, D.A.M., Kostense, S., Sijm, D.T.H.M., and Opperhuizen, A. 1995. Intraspecies variation in lethal body burdens of narcotic compounds. *Aquatic Toxicology (Amsterdam)* 33, 325-342.

van Wezel, A.P., De Vries, D.A.M., Sijm, D.T.H.M., and Opperhuizen, A. 1996. Use of the lethal body burden in the evaluation of mixture toxicity. *Ecotoxicology and Environmental Safety* 35, 236-241.

van Wezel, A.P., and Jonker, M.T.O. 1998. Use of the lethal body burden in the risk quantification of field sediments: Influence of temperature and salinity. *Aquatic Toxicology (Amsterdam)* 42, 287-300.

van Wezel, A.P., and Opperhuizen, A. 1995. Narcosis due to environmental pollutants in aquatic organisms: Residue-based toxicity, mechanisms, and membrane burdens. *Critical Reviews in Toxicology* 25, 255-279.

Veith, G.D., Call, D.J., and Brooke, L.T. 1983. Structure toxicity relationships for the fathead minnow, *Pimephales promelas*: Narcotic industrial chemicals. *Canadian Journal of Fisheries and Aquatic Science* 40, 743-748.

Verdonck, F.A.M., Aldenberg, T., Jaworska, J., and Vanrolleghem, P.A. 2003. Limitations of current risk characterization methods in probabilistic environmental risk assessment. *Environmental Toxicology and Chemistry* 22, 2209-2213.

Versteeg, D.J., Belanger, S.E., and Carr, G.J. 1999. Understanding single species and model ecosystem sensitivity. *Environmental Toxicology & Chemistry* 18, 1329-1346.

Villa, S., Finizio, A., and Vighi, M. 2003. Pesticide Risk Assessment in a Lagoon Ecosystem. Part I: Exposure Assessment. *Environmental Toxicology & Chemistry* 22, 928-935.

Waller, W.T., Ammann, L.P., Birge, W.J., Dickson, K.L., Dorn, P.B., LeBlanc, N.E., Mount, D.I., Parkhurst, B.R., Preston, H.R., Schimmel, S.C., Spacie, A., and Thursby, G.B. 1996. Prediting Instream Effects from WET tests, 271-286. In Grothe, D.R., Dickson, K.L., and Reed-Judkins, D.K., eds., *Whole Effluent Toxicity Testing: An evaluation of Methods adn Prediction of Receiving System Impacts*. SETAC, Pensacola, Florida.

Warne, M., StJ. 1998. Critical Review of methods to derive water quality guidelines for toxicants and a proposal for a new framework., *Supervising Scientist Report 135*. Supervising Scientist, Canberra.

Warne, M., StJ. 2001. Derivation of the Australian and New Zealand water quality guidelines for toxicants. *Australasian Journal of Ecotoxicology* 7, 123-136.

Warne, M., StJ. 2008. Draft recommendations on whole effluent toxicity testing for the Desalination Plant Desal II. CSIRO.

Warne, M., StJ., and Hawker, D.W. 1995. The number of components in a mixture determines whether synergistic and antagonistic or additive toxicity predominate: The funnel hypothesis. *Ecotoxicology and Environmental Safety* 31, 23-28.

Warne, M., StJ., and Westbury, A.-M. 1999. A compliation of toxicity data for chemicals to Australian species. Part II Organic Chemicals. *Australasian Journal of Ecotoxicology* 5, 21-85.

Warren Hicks, W.J., Parkhurst, B.J., and Butcher, J.B. 2002. Methodology for Aquatic Ecological Risk Assessment. In Posthuma, L., Suter, G.W., and Traas, T.P., eds., *Species Sensitivity Distributions in Ecotoxicology*. Lewis, Boca Raton, Florida.

Wharfe, J. 2004. Hazardous chemicals in complex mixtures - A role for direct toxicity assessment. *Ecotoxicology* 13, 413-421.

Wharfe, J., Tinsley, D., and Crane, M. 2004. Managing complex mixtures of chemicals - a forward look from the regulators' perspective. *Ecotoxicology* 13, 485-492.

Wheeler, J.R., Grist, E.P.M., Leung, K.M.Y., Morritt, D., and Crane, M. 2002. Species sensitivity distributions: Data and model choice. *Marine Pollution Bulletin* 45, 192-202.

Widdows, J. 1993. *Marine and Estuarine Invertebrate Toxicity Tests*. Blackwell Scientific Publications, Oxford.

Woodward-Clyde. 1996. *ICI Botany, Groundwater Stage 2 Survey, Contract S2/C3 Water/Soil Phase 2., Sydney, Australia*.

Woodworth, J.G., King, C., Miskiewicz, A.G., Laginestra, E., and Simon, J. 1999. Assessment of the comparative toxicity of sewage effluent from 10 sewage treatment plants in the area of Sydney, Australia using an amphipod and two sea urchin bioassays. *Marine Pollution Bulletin* 39, 174-178.

Zok, S., Boutonnet, J.-C., De Rooij, C., Garny, V., Lecloux, A., Papp, R., Thompson, R.S., and van Wojk, D. 1998. Euro chlor risk assessment for the marine environment Osparcom region: North Sea - chloroform. *Environmental Monitoring and Assessment* 53, 401-424.

Zolezzi, M., Cattaneo, C., and Tarazona, J.V. 2005. Probabilistic ecological risk assessment of 1,2,4-trichlorobenzene at a former industrial contaminated site. *Environmental Science & Technology* 39, 2920-2926.