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Original citation & hyperlink:
Hunt, J, Birch, G, Warne, MSJ & Krassoi, R 2009, 'Direct toxicity assessment of volatile chlorinated hydrocarbon-contaminated groundwater and derivation of a site-specific guideline' Integrated Environmental Assessment and Management, vol. 5, no. 2, pp. 338-348.
https://dx.doi.org/10.1897/IEAM_2008-070.1

DOI 10.1897/IEAM_2008-070.1
ISSN 1551-3777
ESSN 1551-3793

Publisher: Wiley

IEAM_2008-070R
Received 28 August 2008; Accepted 28 December 2008

Direct toxicity assessment of volatile chlorinated hydrocarbon contaminated groundwater and derivation of a site-specific guideline

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Running Title

DTA of VCH Contaminated Groundwater and Guideline Derivation

IEAM_2008-070R
Received 28 August 2008; Accepted 28 December 2008

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 5 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.

KEYWORDS Volatile hydrocarbons Direct toxicity assessment

IEAM_2008-070R
Received 28 August 2008; Accepted 28 December 2008

INTRODUCTION

Historic groundwater contamination with a complex mixture of 14 volatile chlorinated hydrocarbons (VCHs) was identified and extensively characterized at an industrial site in Sydney, Australia (1996). 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

Received 28 August 2008; Accepted 28 December 2008

industrial effluent discharges in the United States (Grothe et al. 1996; USEPA 2000) 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 standardized, 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 1993; Carey et al. 1998), do not interact with nutrients to cause eutrophication, and have not been identified as potential endocrine disruptors (McCarty and Mackay 1993; 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 United States 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

Received 28 August 2008; Accepted 28 December 2008

(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 5 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.

METHODOLOGY

Test Water Preparation

Contaminated groundwater was collected from 2 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 (Figure 1). These 2 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 0.45 µm.

The Number and Selection of Test Species

The toxicity of the contaminated groundwater was assessed using 5 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 represent 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

Received 28 August 2008; Accepted 28 December 2008

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. (2009). 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 summarized 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.

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 h. 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.

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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 fertilization (<1 h). Viable gametes were selected on the basis of fertilization success trials and visual examination of gamete maturity. Eggs were fertilized 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 were selected on the basis of fertilization success trials and visual examination of gamete maturity. Eggs were fertilized 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 fertilization. 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 h. 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 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. 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 10000 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, 3 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. (2009). 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 analyzed by Gas Chromatography Mass Spectrometry (GC/MS)

utilizing 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. 2009). Relationships between percent dilution and concentration of total VCHs for the vials and the jars was presented in Hunt et al. (2009). 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).

Received 28 August 2008; Accepted 28 December 2008

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 3-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 assessments of the BurrliOZ plots indicate 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 2 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 3 scenarios were modeled. 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.

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RESULTS

Chemistry

The composition of groundwater was dominated by 1,2-dichloroethane, which accounted for approximately 90% of the total composition by weight and 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. 2009).

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 4 replicates, 3 reported cell densities of between 5.3×10^4 and 5.7×10^4, while 1 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).

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 5 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

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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 3 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, while 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 Pareto distributions decreased by approximately 3-fold (Additional Scenario 3, Table 5) compared to the original scenario.

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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 favored by some workers because of the strong existing mathematical basis for its interpretation (Duboudin et al. 2004). Despite the various preferences of individuals or organization there is no theoretical basis for assuming the SSD should conform to any particular distribution (Forbes and Forbes 1993). Newman et al. (2000) evaluated a non-parametric bootstrapping methodology, however, the results of this were similar to the log-normal model anyway. Newman et al. (2000) 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 approximately 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 toward 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. (2000) 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 5

species used in the present study, 4 are routinely used test organisms (*N. closterium*, *A. compressa*, *S. commercialis*, and *H. tuberculata*) and 1 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 5 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 5 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 2 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 3 additional scenarios. Increasing the ACR from 5 to 10 (Additional Scenario 1, Table 5), 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, Table 5) and an

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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, Table 5]), the TVs decreased by approximately 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 3-fold 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

Received 28 August 2008; Accepted 28 December 2008

datasets for pesticides suggested that at least 30 data points should be used to minimize variability in derived SSDs, with this number varying between 15 and 55 (Newman et al. 2000). The same review noted that the inability to meet the required sample size to minimize variability does not make the approach invalid, merely results and interpretation should be treated with caution (Newman et al. 2000). 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.

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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 3-fold 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 from DTA on a battery of indigenous test species.

IEAM_2008-070R
Received 28 August 2008; Accepted 28 December 2008

ACKNOWLEDGMENTS

This work was supported by Orica Australia Pty Ltd and ALS Environmental Pty Ltd and was greatly improved by comments from two anonymous reviewers.

IEAM_2008-070R
Received 28 August 2008; Accepted 28 December 2008

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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^{o}C	20±1^{o}C	21 ± 1°C	20 ± 1^{o}C	20 ± 1^{o}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%	

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	44100
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	--	47570

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

-- Denotes that Trigger Values for Total Volatile Chlorinated Hydrocarbons are 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 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

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

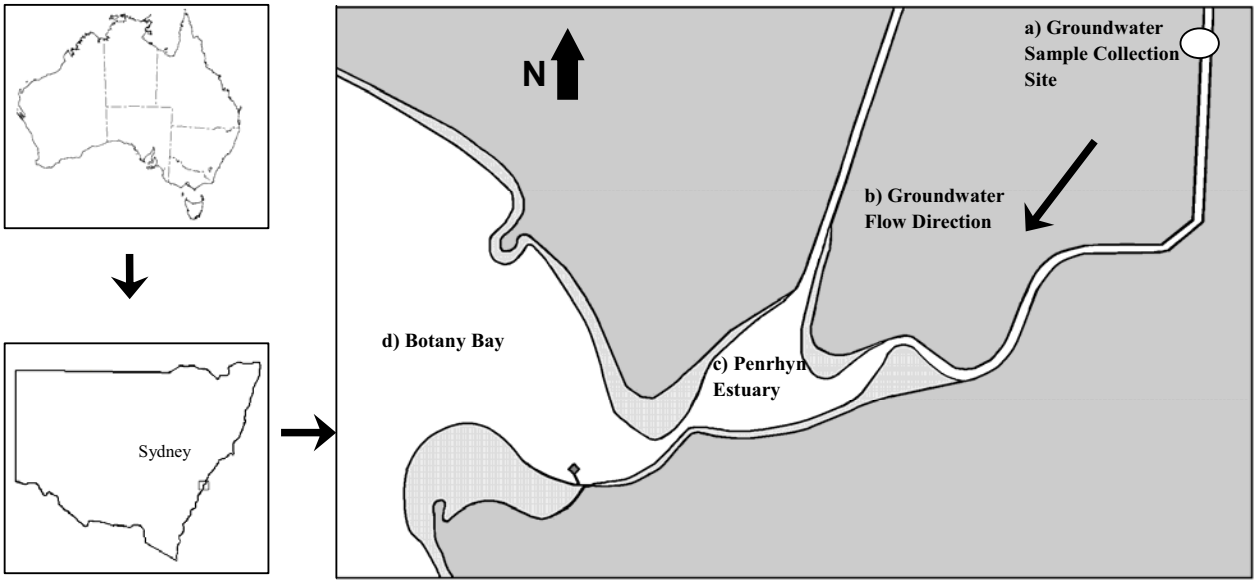

Figure 1. Location plan of Penrhyn Estuary, Sydney, Australia indicating a) the groundwater sample collection site, b) groundwater flow direction and receiving waters in c) Penrhyn Estuary and d) Botany Bay.

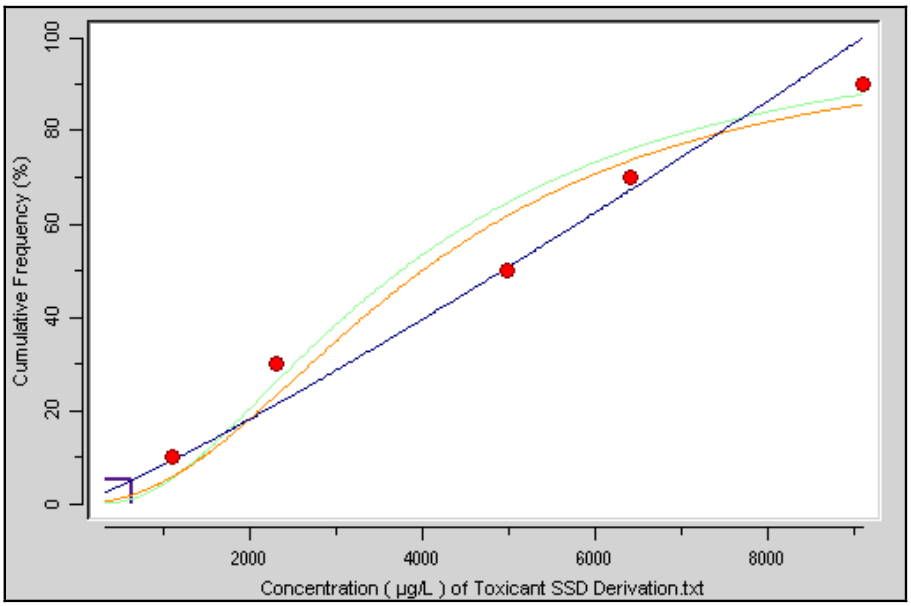

Figure 2. Species sensitivity distributions derived using BurrliOZ™ software for groundwater including the Reciprocal Pareto (r^2=0.84) (blue line), log-normal (r^2=0.88) (green) and log-logistic (orange) distributions. Red circles represent individual NOEC data points.