TOXICITY OF, AND DEVELOPMENT OF PREDICTIVE MODELS FOR, SUBSTITUTED PHENOLS TO *CERIODAPHNIA* CF. *DUBIA* AND *VIBRIO FISCHERI*

Anne-Maree Westbury[1,2,4], Michael StJ Warne[2,3,5*], Richard P Lim[1,2].

[1] Department of Environmental Sciences, University of Technology Sydney (UTS), Westbourne St, Gore Hill NSW 2065, Australia.

[2] DEC/UTS Centre for Ecotoxicology, located at the University of Technology Sydney, Westbourne St, Gore Hill NSW 2065, Australia.

[3] Ecotoxicology and Water Science Section, Department of Environment and Conservation NSW (DEC), PO Box 29, Lidcombe NSW 1825, Australia.

[4] Current address: Victorian Environment Protection Authority, GPO Box 4395QQ, Melbourne, Victoria 3001, Australia.

[5] Current address: CSIRO Division of Land & Water, PMB 2, Glen Osmond SA 5064, Australia.

Manuscript received, 21/12/01; resubmitted, 4/8/03; accepted, 8/8/03.

ABSTRACT

Twelve substituted phenols that have a polar narcotic mode of action had their acute toxicity to the freshwater cladoceran *Ceriodaphnia* cf. *dubia* and the marine bacterium *Vibrio fischeri* determined. The 48-h EC50 (immobilisation) values of the chemicals to *C.* cf. *dubia* ranged from 6.13 to 183 µmol/L while the 30-min EC50 (luminescence) values of the chemicals to *V. fischeri* ranged from 1.39 to 1031 µmol/L. Seventy-five percent of the chemicals were classed as having 'moderate toxicity' to *C.* cf. *dubia* while 25 percent had 'low toxicity'. For *V. fischeri* the percentage of chemicals classified as having 'high', 'moderate' and 'low' toxicity was approximately 17%, 33% and 50% respectively. Quantitative activity-activity relationships (QAARs) that could predict the toxicity of chemicals with a polar narcotic mode of action to *C.* cf. *dubia* were developed by regressing the toxicity data for polar narcotic chemicals to *C.* cf. *dubia* with that of eight non-Australasian species. Those QAARs based on the toxicity data for *Tetrahymena pyriformis*, *Chlorella vulgaris*, *Poecilia reticulata* and *Daphnia magna* were of high quality ($r^2 \geq 0.9$). Only the QAARs for *T. pyriformis*, *C. vulgaris* and *D. magna* had sufficient data to test their validity. This revealed that the absolute percentage difference between experimentally derived EC50 values and those predicted by these QAARs were between 13 and 120%. These QAARs provide an easy, cost-effective means of estimating toxicity values for polar narcotic chemicals to *C.* cf. *dubia*.

Key words: Phenols; QAARs; toxicity models; *Ceriodaphnia* cf. *dubia*; Microtox®

INTRODUCTION

Approximately 60% of industrial chemicals which enter aquatic environments are classified as narcotics as they exert their toxic effect by accumulating in nerve cell membranes and physically interfering with the membrane structure and function (van Wezel *et al.* 1995). Narcotics are divided into two groups according to their mode of action. These are non-polar narcotics (eg. benzenes, saturated aliphatic monoalcohols, saturated monoketones and aromatic hydrocarbons) and the more toxic polar narcotics (eg. most substituted phenols and benzenamines (formerly known as anilines) and pyridines). Extensive reviews of toxicity data for Australasian species conducted by Warne and Westbury (1999) for organic chemicals excluding pesticides (eg. herbicides, insecticides) found very few data for polar narcotics.

There is a general lack of toxicity data for aquatic Australasian species, particularly when compared to the amount available for Northern Hemisphere species (Warne *et al.* 1998, Warne and Westbury 1999; Markich *et al.* 2002). This lack of data poses problems for the derivation of national and site-specific water quality guidelines (WQGs) as well as conducting hazard and risk assessments within Australasia.

Gaps in the aquatic toxicity data for Australasian species have generally been filled using toxicity data for non-Australasian species, particularly from the northern hemisphere (ANZECC 1992; Wu 1996; Warne 1998). For example, the vast majority of toxicity data used to derive the new Australian and New Zealand Water Quality Guidelines (ANZECC and ARMCANZ 2000) were for non-Australasian species. If Australasian species have significantly different inherent sensitivities to non-Australasian surrogate species, or the chemistry of Australasian water is such that the toxicity of chemicals is different, then the WQGs and risk and hazard assessments based on toxicity data for non-Australasian species may either under- or over-protect Australasian species and ecosystems. The potential for over- and/or under-protection arising by using non-indigenous species has been overcome by both the USA (USEPA 1986) and Canada (CCREM 1991) requiring that the majority of data used to derive their WQGs are for species resident in North America. The toxicity data available for Australasian species means that a similar requirement is not practical as this would mean that WQGs for only a very limited number of chemicals could be derived (Warne *et al.* 1998).

There have only been a few studies that have assessed the relative sensitivities of Australasian and non-Australasian species (Johnston *et al.* 1990; Sunderam *et al.* 1992, 1994; Davies *et al.* 1994; Markich and Camilleri 1997; Mulhall 1997; Rose *et al.* 1997). Due to the limited number of studies conducted, the limited number of chemicals used in these comparisons and the inconsistencies of the results, the appropriateness of using toxicity data for non-Australasian species remains unclear.

An alternative to the use of non-Australasian toxicity data, is the use of models that can predict the toxicity of chemicals to Australasian species. There are two major types of such models: Quantitative Structure-Activity Relationships (QSARs), and Quantitative Activity-Activity Relationships (QAARs) that are also

*Author for correspondence, email: michael.warne@csiro.au.

called inter-species conversion equations. QSARs are empirical relationships that use physicochemical properties of compounds to predict the biological activities such as toxicity and bioconcentration, for other related chemicals. QAARs are empirical relationships that model the toxicity of chemicals to one species using either toxicity data of another species, or a different endpoint or measure of toxicity for the same species. In order to derive the first type of QAAR mentioned above, toxicity data for two organisms, to the same set of chemicals, are regressed against each other. Using these QAARs, species for which there is a wide range of toxicity data available, can be used to predict the toxicity of chemicals to species for which there is a limited amount of available data (Kaiser 1998).

Only one set of QAARs has been developed for Australasian species and these modelled the toxicity (ie. 48-h EC50 immobilisation) of non-polar narcotics to the freshwater crustacean *Ceriodaphnia* cf. *dubia* (Rose *et al.* 1997). Due to the lack of toxicity data for polar narcotics to Australasian species (Warne and Westbury 1999) and their prevalence in aquatic environments, it is important to develop QAARs for this group of chemicals.

The aims of this study were: to determine the 48-h EC50 (immobilisation) and 30-min EC50 (luminescence) values of a range of polar narcotic chemicals (substituted phenols) to the Australian cladoceran *Ceriodaphnia* cf. *dubia* and the marine bacterium *Vibrio fischeri* respectively; to determine the relative sensitivity of *C.* cf. *dubia* and non-Australasian aquatic species; and finally to develop and determine the predictive accuracy of QAARs for the toxicity (ie. 48-h EC50 immobilisation) of polar narcotics to *C.* cf. *dubia*.

METHODS

Chemicals

All the test chemicals were analytical reagent grade (> 99% purity) and were used without modification. The test chemicals were 2-cyanophenol, 2-tert-butyl-4-methylphenol, 3-chlorophenol, 3-cyanophenol, 3-fluorophenol, 3-iodophenol, 4-iodophenol, 2,4-dibromophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 3,4,5-trimethylphenol, and methyl-3-hydroxybenzoate.

Toxicity tests

The cladoceran used in this study was identified as *Ceriodaphnia dubia* Richard (Julli *et al.* 1990). While it conforms (cf.) with the taxonomic classification of *C. dubia*, it is morphologically distinct from this species and thus it has been denoted as *C.* cf. *dubia*. The cladocerans were cultured and tested in dechlorinated Sydney mains water that was filtered (1 Ïm), aged (1 month) and adjusted to 500 µS/cm with seawater. The cultures and toxicity tests were maintained in constant temperature cabinets at 23 ± 1ºC with a 16:8 hour light to dark regime and light intensity below 800 Lux at the surface of the test solutions. Cultures of *C.* cf. *dubia* were maintained in 2L glass beakers containing 1.8 L of water and were transferred to fresh water three times weekly. Food was provided after water renewal at a concentration of 25,000 cells/mL of each of the unicellular algae *Pseudokirchneriella subcapitata* Printz (formerly named *Selenastrum capricornutum*) and *Ankistrodesmus* sp.

The NSW EPA static, acute cladoceran toxicity test methodology (Warne and Julli, in prep) was followed. The test containers were 214-mL glass bottles with teflon-lined screw-on lids. The bottles were completely filled removing any headspace in order to minimise volatilisation of the test chemicals.

Each toxicity test consisted of five toxicant concentration treatments arranged in a geometric series, a control and where appropriate a solvent control, each containing at least three replicates. Stock solutions of the chemicals were made on the day of use, using either polished reverse osmosis water or nanograde acetone. The diluent water used in all toxicity tests was the same as that used to maintain the cladocerans. The mean water quality parameters for the diluent water, used in this study, were pH 7.7, free and total chlorine 0.01 and 0.03 mg/L respectively, ammonia 0.01 mg/L, and hardness 65.2 mg/L as $CaCO_3$.

Five cladocerans (< 24 hrs old) were randomly allocated to each container. The temperature, dissolved oxygen, pH and conductivity of the test solutions were measured immediately prior to the addition of the cladocerans and on completion of each test. Cladocerans were not fed during the tests. The tests were terminated after 48 hours and the number of immobile cladocerans, using the ASTM (1988) definition, were counted. Previous work (Rose *et al.* 1997) had shown that there was sufficient dissolved oxygen for five cladocerans to survive in the sealed bottles for periods considerably longer than the test, without any deaths. Tests were considered invalid and the data not included in the study if more than 10% of the control neonates were immobilised.

The 30-min EC50 (luminescence) values of the twelve chemicals to *Vibrio fischeri* were determined using the standard acute Microtox®, method (Microbics Corporation 1988). Aqueous stock solutions of the test chemicals were prepared in volumetric flasks, with the appropriate mass of chemical being weighed out and initially dissolved in methanol. The stock solutions were then diluted with seawater that was osmotically adjusted to a salinity of between 20 - 22 parts per thousand (ppt). Previous work conducted by Mulhall (1997) showed that the maximum percent of methanol in seawater which did not cause any statistically significant ($p<0.05$) reduction in the luminescence of *V. fischeri* after 30 minutes exposure (the maximum duration of the tests conducted) was 1.0%. Therefore, the amount of methanol used to dissolve the chemicals was chosen so that it would be below 1.0% in the highest test concentration. Each Microtox toxicity test consisted of eight serial dilutions (dilution factor of 2) of the toxicant and two sea water controls, with two replicates for each treatment.

The exposure concentrations of the 12 test chemicals were determined for the *C.* cf. *dubia* toxicity tests but not for the *V. fischeri* tests, as the short exposure period and the low temperature at which the Microtox tests were conducted would minimise volatilisation. Chemical analyses were performed on two replicates of each toxicant concentration at the beginning and end of each definitive test. The test chemicals were extracted by two sequential additions of 25 mL of dichloromethane. The dichloromethane was dried using anhydrous sodium sulfate and the volume reduced to approximately 0.5 mL by purging with nitrogen, and then diluted to 1 mL with hexane. Five standards, corresponding to the toxicant concentrations used in the definitive tests, were prepared for each chemical from the stock solutions and were made up to 1 mL with hexane. The 1mL test samples and standards were quantified using either a gas chromatograph (GC) fitted with a ^{63}Ni electron capture detector (ECD) or a gas chromatograph configured with a mass selective

detector (GC-MS). Both instruments were fitted with a capillary column 30 m long and which had a 0.25-mm internal diameter and was coated with 0.25-mm thick DB5 stationary phase. A sample injection volume of 1 mL was used for all the chemical analyses. Initial, final and rates of increasing the temperature varied for the different chemicals.

As the concentrations of the test chemicals were not measured for the *V. fischeri* toxicity tests, nominal concentrations of the test chemicals were used to calculate the concentrations that caused a 50% reduction in bioluminescence after 30 minutes exposure (30-min EC50 (lumin)) were calculated using the Microtox®, M500 programme.

The concentrations used to calculate the toxicity values (48-h EC50 (immob)) for *C.* cf. *dubia* were determined according to the guidance provided by the OECD (1998). For those chemicals that incurred a loss of less than 20%, the measured initial concentrations were used to calculate the EC50 values while for chemicals that suffered greater than 20% loss the geometric means of the initial and final concentrations were used. The 48-h EC50 (immobilisation) values and 95% confidence limits for *C.* cf. *dubia* were determined using the trimmed Spearman-Karber method (Hamilton *et al.* 1977; 1978).

Sources of toxicity data

Wherever possible the toxicity data used to derive the QAARs were obtained from one laboratory using the same experimental conditions, in order to provide a consistent set of data and to optimise the quality of the resulting QAARs. This procedure limited the amount of toxicity data available for *Poecilia reticulata* and *Escherichia coli*. For *P. reticulata* there were two publications with toxicity data for polar narcotics available (Saarikoski and Viluksela, 1982; Benoit-Guyod *et al*. 1984). However, the data in the latter paper were not used in the current study due to their short exposure duration (ie. 24 hours), which was even shorter than that for the *C.* cf. *dubia* and hence were not really comparable. Exceptions were toxicity data for both *C.* cf. *dubia* and *V. fischeri* obtained from the present study and Mulhall (1997) that were conducted in the same laboratory using identical test procedures. The toxicity data used to derive the QAARs are presented in Table 1.

Development of Quantitative-Activity-Activity-Relationships

Eighty percent of the toxicity data for *C. vulgaris, V. fischeri, T. pyriformis, D. magna* and *P. promelas* (Table 1) were randomly selected to form a training set for each species. The chemicals in the training sets were used to develop QAARs. The remaining 20% of the toxicity data for these species became validation sets that were used to test the predictive accuracy of the QAARs. For the remainder of the non-Australasian species (*P. reticulata, P. flesus* and *E. coli*) there were insufficient data (Table 1) to both derive and validate QAARs. For these species all the data were used to derive the QAARs.

The QAARs were developed by regressing the natural logarithm (to base e) of the EC50 (μ mol/L) values for *C.* cf. *dubia* against the natural logarithm of the toxicity (μ mol/L) values for each of the non-Australasian species. A minimum coefficient of determination (r^2) of 0.80 was used to determine QAARs with acceptable modelling capabilities. This cut-off was chosen as QAARs with r^2 values less than 0.80 are unlikely to be able to accurately predict the toxicity values for other chemicals not used to derive the QAAR. However, once this was done the relative quality of the QAARs was determined using Akaikes Informational Criteria (AIC) values, with the quality of the QAARs increasing with decreasing AIC values. The AIC values were calculated using the following equation:

$$AIC = N \cdot \ln S^2 + 2P \qquad (1)$$

where N is the number of experimental points, S is the residual sum of squares of the regression and P is the number of parameters in the QAAR (Reed *et al.* 1984).

Determination of the relative sensitivity of test species

The y-intercept of a QAAR indicates the relative sensitivity of the two test organisms or test systems used to derive it (Rose *et al.* 1997). The more negative the y-intercept the more sensitive is the species that is the dependant variable (y axis) compared to the species that is the independent variable (x axis). The relative sensitivity of two species can be quantified by obtaining the anti-log of the natural logarithm of the y-intercept of the QAAR describing the relationship between the two species.

Validation of QAARs

Generally, a difference between predicted and experimental values of up to one order of magnitude is acceptable for ecotoxicology models such as QSARs (Chiou 1985; McCarty 1986; Blum and Speece 1990; Pawlisz and Peters 1993). However, this is a rather large error. In order to ensure the QAARs developed in this study would provide accurate and useful estimates of toxicity, a mean absolute percentage error of 100% was arbitrarily chosen as the maximum acceptable. QAARs that had mean absolute percentage errors ≤100% were considered 'valid' as they accurately predict toxicity while those with errors > 100% were considered 'invalid'. The mean absolute percentage error for each QAAR was calculated using the following equation:

$$\text{mean absolute \% error} = \sum \left| [(\text{observed EC50i} - \text{predicted EC50i}) / \text{observed EC50i}] \lozenge 100 \right| / n \qquad (2)$$

where observed EC50i is the experimentally measured EC50 value for chemical 'i', predicted EC50i is the EC50 value predicted for chemical 'i' by the QAAR, and n is the number of chemicals in the training set used to derived the QAAR.

RESULTS AND DISCUSSION

Toxicity to *Ceriodaphnia cf. dubia* and *Vibrio fischeri*

The 48-h EC50 (immob) values of the substituted phenols to *C.* cf. *dubia* ranged from 6.13 to 183 μmol/L (Table 1). Based on the Worksafe and NICNAS classification of toxicants (Worksafe and NICNAS 1991) none of the chemicals had 'high toxicity' (EC50 < 1 mg/L), eight (2-tert-butyl-4-methylphenol, 3-chlorophenol, 3-iodophenol, 4-iodophenol, 2,4-dibromophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 3,4,5-trimethylphenol) had 'moderate toxicity' (EC50 between 1 and 10 mg/L) while the remainder (2-cyanophenol, 3-fluorophenol, 3-cyanophenol, methyl-3-hydroxybenzoate) had 'low toxicity' (EC50 > 10 mg/L).

Table 1. *Species, biological endpoints measured, toxicity data (μmol/L) and sources for the data used to develop and validate the quantitative activity-activity relationships (QAARs).*

Chemical	48h EC50 *C. cf.d*	48h EC50 *D. m*	7 and 14 day LC50 *P. r*	96h LC50 *P. p*	96h LC50 *P. f*	6h IGC50 *C. v*	48h IGC50 *T. p*	30min EC50 *V. f*	48h EC50 *E. c*
2-tert-butyl-4-methylphenol	7.31[a]		12.6[d]				50.0[h] (v)	3.56[h]	
3-chlorophenol	76.5[a]				31.1[f]	251[g] (v)	134[h] (v)	124[h]	
3-fluorophenol	95.7[a]					691[g]	415[hi]	627[h]	
3,4,5-trimethylphenol	18.3[a]						117[h]	32.6[h]	
4-iodophenol	37.4[a]					138[g]	140[h] (v)	1.39[h]	
Methyl-3-hydroxybenzoate	101[a]						899[h]	208h[i]	
2,4,5-trichlorophenol	6.13[a]	4.56[c]	6.31[d]		20.3[f]		8.00[h]	12.3[ij]	41.7[i]
3-iodophenol	28.2[a]						76.0[h]	7.66[ij]	
3-cyanophenol	183[a]						1159[h]	325[ij]	
Phenol	176[b]	223[c]	457[d]	346[e]	212[f]	5011[g]	1614[hi]	263[g]	7079[i]
2-bromophenol	55.9[b]	5.20[c] (v)				616[g]	468[hi]	127[g]	758[i]
2-nitrophenol	114[b]	122[c]		1148[e]		39.8[g] (v)	213[h] (v)	238[g] (v2)	794[i]
4-chloro-3-methylphenol	25.9[b]			38.0[e]			160[h]	2.65[g]	
2,4-dichlorophenol	21.8[a]	8.59[c]	33.9[d]	47.9[e]	36.8[f]		92.0[h]	19.8[g]	331[ij]
4-methylphenol	91.2[b]	71.2[c] (v)		151[e]		1659[g]		7.83[g]	263[i]
4-ethylphenol	86.7[b]	46.6[c]		85.1[e]				0.31[g]	
2,4,6-trichlorophenol	8.81[a]	11.1[c]	11.5[d]	24.5[e] (v)	7.00[f]		38.9[h]	137[h] (v1)	191[ij]
2,4-dibromophenol	20.3[b]						40.0[hi]	4.86[i] (v1,2)	
2-cyanophenol	166[a]						925[h]	1031[h] (v1)	
2-chlorophenol	77.2[b]		107[d]	107[e] (v)	54.4[f]	758[g]	656[h]	261[g] (v1,2)	

[a] chemicals tested in the present study, [b] Mulhall (1997), [c] Kuhn *et al.* (1989), [d] Saarikoski and Viluksela (1982), [e] Bearden and Schultz (1997), [f] Smith *et al.* (1994), [g] Jaworska and Schultz (1991), [h] Cronin and Schultz (1996), [i] Jaworska and Schultz (1994).

(v) validation set, (v1) chemical belongs to the first validation set for the *V. fischeri* QAAR, (v2) chemical belongs to the second validation set for the *V. fischeri* QAAR, (v1,2) chemical belongs to both the first and second validation sets for the *V. fischeri* QAAR. *C.* cf. *d* - *Ceriodaphnia* cf. *dubia* (cladoceran), *D. m* - *Daphnia magna* (cladoceran), *P. r* - *Poecilia reticulata* (guppy), *P. p* - *Pimephales promelas* (Fathead Minnow), *P. f* - *Platichthys flesus* (flounder), *C. v* - *Chlorella vulgaris* (algae), *T. p* - *Tetrahymena pyriformis* (ciliate), *V. f* - *Vibrio fischeri* (marine bacteria), *E. c* - *Escherichia coli* (bacteria).

The 30-min EC50 (lumin) values of the chemicals to *V. fischeri* ranged from 1.39 to 1031 µmol/L (Table 1). There were two chemicals (4-iodophenol, 2-tert-butyl-4-methylphenol) that had 'high toxicity', four chemicals (2,4-dibromophenol, 3-iodophenol, 2,4,5-trichlorophenol and 3,4,5-trimethylphenol) had 'moderate toxicity' while six chemicals (3-chlorophenol, 2,4,6-trichlorophenol, methyl-3-hydroxybenzoate, 3-cyanophenol, 3-fluorophenol and 2-cyanophenol) had 'low toxicity'.

Phenol is the parent compound of the twelve chemicals tested in the current study. Thus, by comparing the toxicity values of phenol (48-h EC50 (immob) and 30-min EC50 (lumin)) and the test chemicals using the standard error of the difference test (Sprague and Fogels 1977), the effect of the type, position and number of substituents can be determined. All the substituents, except 2-cyano and 3-cyano increased the toxicity of the test chemicals to *C.* cf. *dubia* and *V. fischeri* compared to phenol. For *C.* cf. *dubia* the toxicity values of the 2-cyanophenol (EC50 = 166 µmol/L) and 3-cyanophenol (EC50 = 183 µmol/L) were not significantly ($p > 0.05$) different to that for phenol (EC50 = 176 µmol/L). For *V. fischeri* the 2-cyanophenol and 3-cyanophenol were significantly ($p < 0.05$) less toxic than phenol (Table 1).

The toxicity values of the various substituted phenols were also compared using the standard error of the difference test (Sprague and Fogels 1977). This indicated that for both *C.* cf. *dubia* and *V. fischeri* bromo-substituted phenols were more toxic than the chloro-substituted (ie. 2-bromophenol > 2-chlorophenol and 2,4-dibromophenol > 2,4-dichlorophenol) and 3-iodophenol was more toxic than 3-chlorophenol. These findings are consistent with those of Liu *et al.* (1996) who found that the toxicity of substituted compounds decreased in the order iodine, fluorine, bromine and chlorine. The only exception to the order of decreasing toxicity developed by Liu *et al.* (1996) observed in the present study, was that 3-chlorophenol was more toxic than 3-fluorophenol for both species.

In the present study 3-chlorophenol was more toxic than 2-chlorophenol for both *C.* cf. *dubia* and *V. fischeri*. This result agrees with the findings of Ravanel *et al.* (1985) and Kishino and Kobayashi (1996), where phenolic compounds with chlorine substituents in the ortho (2-) position, were found to be less toxic than compounds with chlorine substituents in other positions on the benzene ring. Shannon *et al.* (1991) and Kishino and Kobayashi (1996) proposed that this decrease in toxicity of ortho-positioned chlorophenols is due to hydrogen bonding between the substituent and the hydroxyl (OH) group. It has been suggested that the resulting steric (Hansch and Leo 1995) or ionisation (Shannon *et al.* 1991) effects decrease the ability of the OH group of phenols to interact with the target site.

The number of substituents was also seen to affect the toxicity of the chemicals in this study, with toxicity decreasing in the order of tri-, di-, and mono-substituted compounds. This was observed for *C.* cf. *dubia* with 2,4,6-trichlorophenol > 2,4-dichlorophenol > 2-chlorophenol; 2,4,6-tribromophenol > 2,4-dibromophenol > 2-bromophenol; and 3,4,5-trimethylphenol > 3,5-dimethylphenol. Smith *et al.* (1994) and Kishino and Kobayashi (1996) both also found the toxicity of chlorophenols decreased in the order of trichloro > dichloro > monochloro substituted compounds. The same trend was generally observed for the toxicity of chemicals to *V. fischeri* however there were some exceptions. The exceptions were some of the tri-substituted phenols. For example, 2,4-dichlorophenol was more toxic than 2-chlorophenol but also much more toxic than 2,4,6-trichlorophenol. Similarly, 2,4-dibromophenol was more toxic than 2-bromophenol but also much more toxic than 2,4,6-tribromophenol. One explanation is that the 2,4,6-tribromophenol and 2,4,6-trichlorophenol do not act by a polar narcosis mode of action but rather by the much less toxic non-polar narcosis mode of action. Later analysis in this paper found this to be the case for 2,4,6-trichlorophenol. It is therefore possible that the 2,4,6-tribromophenol also acts by the same mode of action.

Relative sensitivities of the test systems

Ceriodaphnia cf. *dubia* and *D. magna* were tested under essentially identical experimental conditions, with 48-h immobilisation as the test endpoint for both species. Therefore, the comparison of the sensitivity of these two species using the antilog of the natural logarithm of the y-intercept of the QAAR between the species (Table 2), is a comparison of their inherent sensitivities. The *D. magna* were more inherently sensitive than *C.* cf. *dubia* by a factor of approximately 1.4 (Table 2).

For all other species the biological endpoint and experimental conditions differed to those used for *C.* cf. *dubia*. The comparison of the toxicity data for these species to *C.* cf. *dubia* was therefore a comparison of the relative sensitivity of the test systems, which is a combination of inherent sensitivity, sensitivity of the endpoints and sensitivity due to variations in experimental conditions. The relative sensitivity of the *P. flesus*, *T. pyriformis* and *P. reticulata* test systems were approximately the same as that of *C.* cf. *dubia*. The *E. coli*, *D. magna*, *C. vulgaris*, *P. promelas* and *V. fischeri* test systems were 1.3, 1.4, 2.0, 2.0, and 3.8 times more sensitive respectively than *C.* cf. *dubia* (Table 2). Therefore, the relative sensitivity of all these test systems to polar narcotics increased in the following order: *P. flesus* < *P. reticulata* < *C.* cf. *dubia* < *T. pyriformis* < *E. coli* < *D. magna* < *C. vulgaris* < *P. promelas* < *V. fischeri*.

A similar study by Rose *et al.* (1997) on non-polar narcotic chemicals found the relative sensitivity of test systems to non-polar narcotics increased in the following order: *P. promelas*, *V. fischeri*, *P. reticulata*, *D. magna* and *C.* cf. *dubia*. While this order is very different to that for the polar narcotics found in the current study, there are two noteworthy differences. Firstly, *C.* cf. *dubia* went from being more sensitive to non-polar narcotics than *P. promelas*, *V. fischeri*, *P. reticulata* and *D. magna* to being the least sensitive of them all for polar narcotics. Secondly, *V. fischeri* had a low sensitivity to non-polar narcotics but was the most sensitive to polar narcotics.

Rose *et al.* (1997) found *D. magna* to be 4.3 times less inherently sensitive to non-polar narcotics than *C.* cf. *dubia* whereas, the present study showed *D. magna* to be approximately twice as sensitive to polar narcotics as *C.* cf. *dubia*. Mulhall (1997) found that *D. magna* was on average 1.45 times more sensitive than *C.* cf. *dubia* to substituted phenols and benzenamines that had polar narcotic and oxidative uncoupler mechanisms of action. The results of these three studies clearly indicate that *D. magna* underestimates and overestimates the sensitivity of *C.* cf. *dubia* to non-polar and polar narcotics respectively. These differences are important as *D. magna* is often used as a surrogate species for Australasian cladoceran species to develop water quality guidelines (eg. ANZECC and ARMCANZ 2000) and to conduct both hazard and

Table 2. *QAARs and the corresponding statistical information for toxicity data of polar narcotics to the Australian cladoceran* Ceriodaphnia *cf.* dubia *(Y) and non-Australasian species arranged in order of decreasing quality and the sensitivity of each species relative to* C. *cf.* dubia. *AIC = Akaikes Informational Criteria.*

QAAR	r^2	P	Df	AIC	Sensitivity relative to *C.* cf. *dubia*[1]
$Y = 0.681\ logIGC50_{tp} + 0.051$	0.92	<0.001	13	-41.27	1.1
$Y = 0.418\ logICG50_{cv} + 0.675$	0.90	0.004	5	-41.21	2.0
$Y = 0.848\ logLC50_{pr} + 0.047$ *	0.98	<0.001	5	-38.23	1.0
$Y = 0.858\ logEC50_{dm} + 0.301$	0.91	0.003	5	-18.94	1.4
$Y = 0.517\ logLC50_{pp} + 0.707$	0.63	0.053	5	-14.85	2.0
$Y = 0.872\ logLC50_{lr} + 0.532$ *	0.73	0.029	5	-13.27	1.7
$Y = 0.691\ logEC50_{ec} + 0.242$ *	0.70	0.019	6	-7.46	1.3
$Y = 0.995\ logLC50_{pf} - 0.024$ *	0.70	0.092	5	-6.11	1.0
$Y = 0.230\ logEC50_{vf} + 1.34$	0.25	0.031	15	28.75	3.8

[1] a value of 1 means the species have the same sensitivity, while larger values mean the species is that many times more sensitive than *C.* cf. *dubia*.

* - QAARs which could not be validated. pr - *P. reticulata*; tp - *T. pyriformis*; dm - *D. magna*; cv - *C. vulgaris*; pf -*P. flesus*; ec - *E. coli*; pp - *P. promelas*; vf - *V. fischeri*.

ecological risk assessments. The extent of effect that this would have will depend on whether or not correcting the toxicity data to estimate the toxicity to *C.* cf. *dubia* affects the distribution of the cumulative frequency of species toxicity data. It is not possible to generalise about the effect that using *C.* cf. *dubia* data rather than *D. magna* data would have as it would most likely differ for each chemical.

QAARs developed

The QAARs developed in this study are presented in Table 2. Only four of the eight QAARs developed were deemed acceptable ($r^2 > 0.80$). These were in order of decreasing quality (ie. increasing AIC values) *T. pyriformis*, *C. vulgaris*, *P. reticulata* and *D. magna* (Table 2). These QAARs all differ from each other in having quite different gradients and/or y-intercepts (Table 2).

In comparison, Rose *et al.* (1997) found high quality non-polar narcotic QAARs for *P. reticulata* (AIC = -38.23, $r^2 = 0.98$), *D. magna* (AIC = -18.94, $r^2 = 0.91$), *P. promelas* (AIC = -11.13, $r^2 = 0.88$) and *V. fischeri* (AIC = 45.9, $r^2 = 0.91$). They could not derive QAARs for either *C. vulgaris* or *T. pyriformis* because there were no suitable toxicity data.

Rose *et al.* (1997) also found a high quality relationship (AIC = -11.13, r^2 =0.88) between *C.* cf. *dubia* and *P. promelas* for non-polar narcotics, while the corresponding QAAR for polar narcotics derived in the present study was not statistically significant ($p>0.05$) and of considerably lower quality (AIC = -14.85, $r^2 = 0.65$).

Another difference between the results obtained in this study and those by Rose *et al.* (1997) was the quality of the *V. fischeri* QAAR. In the current study this relationship was very weak (AIC = 28.75, $r^2 = 0.29$) while Rose *et al.* (1997) found this relationship to be of high quality (AIC = 45.9, $r^2 = 0.84$). Cronin and Dearden (1993) and Kaiser (1998) similarly obtained strong relationships between *P. promelas* and *V. fischeri* for non-polar narcotics, but relatively weak relationships for chemicals with more specific modes of action including polar narcosis.

It has been proposed that *V. fischeri* does not effectively model polar narcosis, because only some of the chemicals shown to operate by polar narcosis in species from higher taxa operate by the same mode of action in *V. fischeri*, with the remainder exhibiting a non-polar narcotic mode of action (Kamlet *et al.* 1986; Cronin *et al.* 1991; Cronin and Dearden 1993; Jaworska and Schultz 1994). The fact that some of the test chemicals may have been operating by the non-polar narcotic mode of action in *V. fischeri* might explain the weak relationship found between *V. fischeri* and *C.* cf. *dubia* in this study (Table 2). This hypothesis was tested by identifying the mode of action of the test chemicals in *V. fischeri* and repeating the QAAR analysis using only those chemicals identified as polar narcotics.

The mode of action of the chemicals in the *V. fischeri* QAAR was identified using the excess toxicity (Te) method developed by Lipnick *et al.* (1987). The excess toxicity (Te) for each test chemical was calculated using the following equation:

Te = toxicity value predicted by non-polar narcotic QAAR / observed value (3)

where the toxicities of the test chemicals as non-polar narcotics were calculated using a QAAR for known non-polar narcotics relating *C.* cf. *dubia* and *V. fischeri* that was developed by Rose *et al.* (1997).

According to the excess toxicity method, Te values greater than 2 or less than 2 indicate the chemical exerts its toxic effect by a more specific mode of action than non-polar narcosis (ie. polar narcosis) (Lipnick *et al.* 1987). Three of the twenty chemicals for which *V. fischeri* toxicity data were available (Table 1) were identified as

Table 3. *Percentage errors between the predicted and measured 48-h EC50 (immobilisation) values for* Ceriodaphnia *cf.* dubia *and corresponding mean absolute % errors, for the QAARs with r^2 values greater than 0.80.*

Non-Australasian Species	Chemical (% error between predicted and observed toxicity values)	Mean absolute % error
Daphnia magna	4-methylphenol (14.7)	50.0
	2-bromophenol (85.3)	
Chlorella vulgaris	3-chlorophenol (38.0)	59.3
	2-nitrophenol (80.7)	
Tetrahymena pyriformis	4-iodophenol (13.0)	63.5
	3-chlorophenol (58.7)	
	2-nitrophenol (61.9)	
	2-tert-butyl-4-methylphenol (120.5)	

non-polar narcotics in *V. fischeri*. These were 2,4,6-trichlorophenol (Te = 0.52), 2-cyanophenol (Te = 1.58) and 3-fluorophenol (Te = 1.88). Of these 3-fluorophenol belonged to the training set while the 2-cyanochlorophenol and 2,4,6-trichlorophenol belonged to the validation set originally established (indicated by v1 in Table 1). These chemicals were removed. In order to maintain the 80:20 ratio between data in the training and validation sets, one chemical was randomly selected (2-nitrophenol) and removed from the training set and added to the validation set (indicated by v2 in Table 1). The QAAR was recalculated and the following obtained:

$$\text{Log EC50}_{cd} = 0.21 \log \text{EC50}_{vf} + 1.35 \qquad r^2 = 0.19, df = 13 \quad (4)$$

The exclusion of non-polar narcotic chemicals marginally decreased the quality (r^2) of the QAAR. This indicates at least for the data available in this study, that the inclusion of chemicals operating by a non-polar narcosis mode of action in the *V. fischeri* QAAR was not the cause of the inability of this QAAR to accurately model the toxicity data of polar narcotics to *C.* cf. *dubia*. Other factors must be responsible for the inability of *V. fischeri* to model polar narcosis in *C.* cf. *dubia* however, identification of these factors is outside the scope of this paper. The results of the present study support the conclusion of Jaworska and Schultz (1994) that *V. fischeri* can not be used reliably to compare the mode of action or predict the toxicity to other species for polar narcotics.

Validation of QAARs

Of the eight QAARs developed (Table 2) only five, those for *D. magna*, *C. vulgaris*, *T. pyriformis*, *P. promelas* and *V. fischeri*, had sufficient toxicity data to be validated. The predictive accuracy of the QAARs that contained enough data and were of acceptable quality, was determined by calculating the mean absolute % error between the experimental and QAAR-predicted EC50 values (µmol/L) (Table 3). Given the small number of chemicals in the validation sets the mean absolute % errors should only be taken as indicative of the predictive accuracy.

The three QAARs of acceptable quality ($r^2 > 0.80$) for *D. magna*, *C. vulgaris* and *T. pyriformis* were all able to predict the 48-h EC50 (immob) values of the chemicals in the validation sets to *C.* cf. *dubia*, with absolute % errors ranging from 13 – 120% (Table 3). This is particularly good considering the validation sets for *D. magna*, *C. vulgaris* and *T. pyriformis* contained chemicals with 14, 6 and 4 fold differences respectively, between the highest and lowest EC50 values.

The QAARs for *P. promelas* and *V. fischeri* were not validated as their abilities to model *C.* cf. *dubia* toxicity data were low with r^2 values of 0.63 and 0.25 (Table 2) respectively.

The QAAR for *P. reticulata* could not be validated due to insufficient toxicity data being available. Because this QAAR had a similar quality (AIC = -38.23, $r^2 = 0.98$) (Table 2) as those for *D. magna*, *C. vulgaris* and *T. pyriformis* one might expect a similar level of accuracy in predicting the toxicity values of the chemicals in the validation set. However, it can not be assumed that it will accurately predict the toxicity values of other chemicals. In fact, the accuracy of the predictions of this QAAR and the others that could not be validated is unknown.

D. magna most accurately predicted the toxicity values of polar narcotics to *C.* cf. *dubia* with absolute % errors of 14.7 and 85.3% (mean 50%) between the QAAR predicted and measured toxicity values (Table 3). Similarly, *D. magna* was shown by Rose *et al.* (1997) to very accurately predict the 48-h EC50 (immob) values of non-polar narcotics to *C.* cf. *dubia*, having a mean absolute % error of 29.9%. Studies by Le Blanc (1984), Suter *et al.* (1983) and Sloof *et al.* (1986) found stronger correlations between toxicity data for more closely related taxa than for more distantly related species. The close taxonomic relationship between *C.* cf. *dubia* and *D. magna* (i.e. they are both cladocerans) may explain why *D. magna* was best able to predict the toxicity data for chemicals to *C.* cf. *dubia*.

Usefulness and Limitations of the QAARs

A major advantage of being able to use the species *D. magna* and *T. pyriformis* for the prediction of the toxicity of polar narcotic chemicals to *C.* cf. *dubia*, is that for both species there is a wide range of toxicity data for these chemicals available compared to other species. This means that the 48-h EC50 (immob) values for a large number of polar narcotics to *C.* cf. *dubia* can be estimated from these two QAARs. The other species for which there is a large amount of toxicity data is *V. fischeri*. It is therefore unfortunate that the quality of the *V. fischeri* QAAR was so low (AIC = 28.75, $r^2 = 0.25$).

A limited number of chemicals was used in the development (ie. 6) and validation (ie. 2) of the QAARs for *D. magna* and *C. vulgaris*. The number of chemicals used to develop the QAARs and therefore the robustness of the QAARs, could have been increased by not validating the QAARs. However, it was viewed as being of greater importance to produce models for which there was a measure of their predictive accuracy. Given the accurate predictions exhibited in the present study for these QAARs, it would therefore be beneficial to expand the datasets and develop more robust QAARs for these two species.

Potential users of the QAARS developed in this study should be aware of several limitations. These are that the QAARs:

1. are only valid for polar narcotic chemicals;
2. can only predict the 48-h EC50 (immobilisation) values for *C.* cf. *dubia* under the experimental conditions used in this study; and
3. should only use the following types of polar narcotic toxicity data: 48-h EC50 (immob) data for *D. magna*, 6-h IGC50 (population growth) data for *C. vulgaris* and 48-h IGC50 (population growth) data for *T. pyriformis*. In addition, the toxicity data can only be used if they lie within the range of toxicity values used to derive the QAARs.

The first limitation means that it must be determined whether or not a chemical is a polar narcotic. A definite classification of whether a chemical is a polar narcotic should be made by referring to either the OECD (1992) or Verhaar *et al.* (1992).

Despite these limitations, QAARs could play a significant role in risk and hazard assessment, as they can provide estimates of the toxicity of chemicals when experimentally derived values are lacking. The QAARs developed in this study use the comparatively abundant toxicity data for non-Australasian species to estimate the 48-h EC50 (immobilisation) values of polar narcotics to *C.* cf. *dubia*. The advantages of these QAARs are that they are easy to use and cost-effective compared to toxicity tests. The success of these relationships indicates that it may be possible to develop QAARs to predict the toxicity of other groups of chemicals to native Australasian species.

CONCLUSIONS

The 48-h EC50 (immobilisation) and 30-min EC50 (luminescence) values of twelve substituted phenols ranged from 6.13 to 183 µmol/L and from 1.39 to 1030 µmol/L for *C.* cf. *dubia* and *V. fischeri* respectively. The relative sensitivity to polar narcotics of the test systems used in the quantitative-activity-activity relationship (QAAR) analysis increased in the following order: *P. flesus*, *C.* cf. *dubia*, *P. reticulata*, *T. pyriformis*, *E. coli*, *D. magna*, *C.* v*ulgaris*, *P. promelas*, and *V. fischeri*. The inherent sensitivity of *D. magna* was twice that of *C.* cf. *dubia*. Four QAARs were developed that were of an acceptable quality (r^2 values > 0.80). Of these only the QAARs based on *T. pyriformis*, *D. magna* and *C.* v*ulgaris* had sufficient data to permit validation. All three QAARs predicted the 48-h EC50 (immob) values of polar narcotics to *C.* cf. *dubia* with absolute % errors between values predicted by these QAARs and those measured experimentally of between 13 and 120%. These QAARs can be used to predict the 48 hour EC50 (immob) values of polar narcotics to *C.* cf. *dubia* from 48 hour EC50 (immob) data for *D. magna*; 6 hour IGC50 (growth) data for *C.* v*ulgaris*; and 48 hour IGC50 (growth) data for *T. pyriformis*.

REFERENCES

ANZECC (Australian and New Zealand Environment and Conservation Council). 1992. *Australian Water Quality Guidelines for Fresh and Marine Waters.* ANZECC, Canberra, Australia.

ANZECC and ARMCANZ (Australian and New Zealand Environment and Conservation Council and the Agriculture and Resource Ministers Council of Australia and New Zealand) 2000. *Australian and New Zealand guidelines for fresh and marine water quality*. ANZECC and ARMCANZ, Canberra, Australia.

ASTM (American Society for Testing and Materials). 1988. *Standard Guide for Conducting Acute Toxicity Tests on Aqueous Effluents with Fishes, Macroinvertebrates, and Amphibians.* American Society for Testing and Materials. Designation E1192.

Bearden AP and Schultz TW. 1997. Structure-activity relationships for *Pimephales* and *Tetrahymena*: A mechanism of action approach. *Environ. Toxicol. Chem.* **16**, 1311-1317.

Benoit-Guyod JL, Andre C, Taillandier G, Rochat J and Boucherle, A. 1984. Toxicity and QSAR of chlorophenols on *Lebistes reticulatus*. *Ecotoxicol. Environ. Saf.* **8**, 227-235.

Blum DJW and Speece RE. 1990. Determining chemical toxicity to aquatic species. *Environ. Sci. Technol.* **24**, 284-293.

CCREM (Canadian Council of Resource and Environment Ministers). 1991. *Canadian water quality guidelines.* Canadian Council of Resource and Environment Ministers, Inland Water Directorate. Environment Canada, Ottawa, Canada.

Chiou CT. 1985. Partition co-efficients of organic compounds in lipid-water systems and correlations with fish bioconcentration. *Environ. Sci. Technol.* **19**, 57-62.

Cronin MTD, Dearden JC and Dobbs AJ. 1991. QSAR studies of comparative toxicity in aquatic organisms. *Sci. Total Environ*. **109/110**, 431-439.

Cronin MTD, Dearden JC. 1993. The comparative toxicology of the Microtox®, test. In *Ecotoxicology Monitoring*, Richardson, M. (Ed), VCH., Weinheim, Germany. pp 213-223.

Cronin MTD and Schultz TW. 1996. Structure-toxicity relationships for phenols to *Tetrahymena pyriformis*. *Chemosphere* **32**, 1453-1468.

Davies PE, Cook LSJ and Goenarso D. 1994. Sublethal responses to pesticides of several species of Australian freshwater fish and crustaceans and rainbow trout. *Environ. Toxicol. Chem.* **13**, 1253-1262.

Hamilton MA, Russo RC and Thurston RV. 1977. Trimmed Spearman-Karber Method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* **11**, 714-719. Correction. 1978. **12**, 417.

Hansch C and Leo A. 1995. *Exploring QSAR: Fundamentals and Applications in Chemistry and Biology.* American Chemical Society, USA.

Jaworska JS and Schultz TW. 1991. Comparative toxicity and structure-activity in *Chlorella* and *Tetrahymena*: Monosubstituted phenols. *Bull. Environm. Contam. Toxicol*. **47**, 57-62.

Jaworska JS and Schultz TW. 1994. Mechanism-based comparisons of acute toxicities elicited by industrial organic chemicals in Procaryotic and Eucaryotic systems. *Ecotoxicol. Environ. Saf.* **29**, 200-213.

Johnston N, Skidmore J and Thompson GB. 1990. *Applicability of OECD data to Australian aquatic species.* A Report to the Advisory Committee on Chemicals in the Environment, Australian and New Zealand Environment Council. 126 pp.

Julli M, Chapman J and Thompson GB. 1990. Use of Australian cladocerans to generate life-cycle toxicity data. *Environ. Monitor. Assess.* **14**, 353-362.

Kaiser KLE. 1998. Correlations of *Vibrio fischeri* bacteria test data with bioassay data for other organisms. *Environ. Health Perspect*. **106**, 583-591.

Kamlet MJ, Doherty RM, Veith GD, Taft RW and Abraham MH. 1986. Solubility properties in polymers and biological media. 7. An analysis of toxicant properties that influence inhibition of bioluminescence in *Photobacterium phosphoreum* (the Microtox Test). *Environ. Sci. Technol*. **20**, 690-695.

Kishino T and Kobayashi K. 1996. Acute toxicity and structure-activity relationships of chlorophenols in fish. *Water Research* **30**, 387-392.

Kuhn R, Pattard M, Pernak KD and Winter A. 1989. Results of the harmful effects of selected water pollutants (anilines, phenol, aliphatic compounds) to *Daphnia magna*. *Water Res.* **23**, 495-499.

Le Blanc G. 1984. Interspecies relationships in acute toxicity of chemicals to aquatic organisms. *Environ. Toxicol. Chem*. **3**, 47-60.

Lipnick RL, Watson KR and Strausz AK. 1987. A QSAR study of the acute toxicity of some industrial organic chemicals to goldfish. Narcosis, electrophile, and protoelectrophile toxicity mechanisms. *Xenobiotica* **17**, 1011-1025.

Liu ZT, Wang LS, Chen SP, Li W and Yu HX. 1996. Analysis and prediction of structure-reactive toxicity relationships of substituted aromatic compounds. *Bull. Environ. Contam. Toxicol.* **57**, 421-425.

Markich SJ and Camilleri C. 1997. *Investigation of metal toxicity to tropical biota: Recommendations for revision of the Australian Water Quality Guidelines*. A Report to the Australian and New Zealand Environment and Conservation Council, Project 95/7. 91p.

Markich SJ, Warne MStJ, Westbury A-M and Roberts CJ. 2002. A compilation of toxicity data for chemicals to Australasian aquatic species. Part 3: Metals and Metalloids. *Australas. J. Ecotoxicol.* **8**, 1-137.

McCarty LS. 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. *Environ. Toxicol. Chem.* **5**, 1071-1080.

Microbics Corporation 1988. *How to run a standard Microtox test*. part 555880, Release 2. Carlsbad, CA.

Mulhall A. 1997. *Models to predict the toxicity of selected phenols and benzenamines to a cladoceran and a marine bacterium*. Honours Thesis, University of Technology Sydney, Sydney, New South Wales, Australia.

OECD (Organisation for Economic Co-operation and Development). 1992. *Report of the OECD workshop on Quantitative Structure-Activity Relationships (QSARs) in aquatic effects assessment*. OECD Environment Monographs No. 58, OECD, Paris, 95 pp.

OECD (Organisation for Economic Co-operation and Development). 1998. Daphnia magna *reproduction test*. OECD, Paris, 22 pp.

Pawlisz AV and Peters RH. 1993. A test of the equipotency of internal burdens of nine narcotic chemicals using *Daphnia magna*. *Environ. Sci. Technol.* **27**, 2801-2806.

Ravanel P, Taillandier G, Tissut M and Benoit-Guyod JL. 1985. Effect of chlorophenols on isolated plant mitochondria activities: A QSAR study. *Ecotoxicol. Environ. Saf.* **9**, 300-320.

Reed K, Mereish K and Jensen B. 1984. Quantitative relationships between structure and pharmakokinetic parameters using molecular connectivity chi indices. 1. Substituted 2-sulfapyridines. *J. Pharm. Sci.* **73**, 237-240.

Rose R, Warne MStJ and Lim R. 1997. Inter-species conversion equations for predicting the toxicity of non-polar narcotic chemicals to *Ceriodaphnia* cf. *dubia*. *Australas. J. Ecotoxicol*. **3**, 75-83.

Saarikoski J and Viluksela M. 1982. Relation between physicochemical properties of phenols and their toxicity and accumulation in fish. *Ecotoxicol. Environ. Saf.* **6**, 501-512.

Shannon RD, Boardman GD and Dietrich AM. 1991. Mitochondrial response to chlorophenols as a short-term toxicity assay. *Environ. Toxicol. Chem.* **10**, 57-66.

Slooff W, van Oers JAM and De Zwart D. 1986. Margins of uncertainty in ecotoxicological hazard assessment. *Environ. Toxicol. Chem.* **5**, 841-852.

Smith S, Furay VJ, Layiwola PJ and Menezes-Filho JA. 1994. Evaluation of the toxicity and quantitative structure-activity relationships (QSAR) of chlorophenols to the copepodid stage of a marine Copepod (*Tisbe battagliai*) and two species of benthic flatfish, the flounder (*Platichthys flesus*) and sole (*Solea solea*). *Chemosphere* **28**, 825-836.

Sprague JB and Fogels A. 1977. *Watch The Y In Bioassay*. Proc. 3rd Aquatic Toxicity Workshop, Halifax, N.S., NoV. 2-3 1976. Environmental Protection Service Technical Report No. EPS-5-AR-77-1, Halifax, Canada.

Sunderam RIM, Cheng DMH and Thompson GB. 1992. Toxicity of endosulfan to native and introduced fish in Australia. *Environ. Toxicol. Chem*. **11**, 1469-1476.

Sunderam RIM, Thompson GB, Chapman JC and Cheng DMH. 1994. Acute and chronic toxicity of endosulfan to two Australian cladocerans and their applicability in deriving water quality criteria. *Arch. Environ. Contam. Toxicol.* **27**, 541-545.

Suter GWI, Vaughan DS and Gardner RH. 1983. Risk assessment by analysis of extrapolation error, a demonstration for effects of pollutants on fish. *Environ. Toxicol. Chem.* **2**, 369-378.

USEPA (United States Environmental Protection Agency). 1986. *Quality criteria for water*. EPA Report No. EPA 440/5 86-001. US Department of Commerce, Virginia, USA.

van Wezel AP, Punte SS and Opperhuizen A. 1995. Lethal body burdens of polar narcotics: Chlorophenols. *Environ. Toxicol. Chem.* **14**, 1579-1585.

Verhaar HJM, Van Leeuwen CJ and Hermens JLM. 1992. Classifying environmental pollutants. 1 : Structure-activity relationships for prediction of aquatic toxicity. *Chemosphere* **25**, 471-491.

Warne MStJ. 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, ACT, Australia.

Warne MStJ and Julli M. In prep. Protocol for acute and chronic toxicity tests using the cladocerans *Ceriodaphnia* cf. *dubia* Richard and *Daphnia carinata* King.

Warne MStJ, Westbury A-M and Sunderam R. 1998. A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides. *Australas. J. Ecotoxicol*. **4**, 93-144.

Warne MStJ and Westbury A-M. 1999. A compilation of toxicity data for chemicals to Australasian aquatic species. Part 2: Organic chemicals. *Australas. J. Ecotoxicol*. **5**, 21-84.

Worksafe and NICNAS. 1991. *Selection procedures for priority existing chemicals*. Worksafe Australia, Sydney, Australia, 52 pp.

Wu R. 1996. Ecotoxicology: problems and challenges in Australasia. *Australas. J. Ecotoxicol.* **2**, 1.