CONSIDERATIONS WHEN APPLYING THE REVISED TOXICANT GUIDELINES

John C Chapman*, Michael StJ Warne and Ron WR Patra
Ecotoxicology Section, Environment Protection Authority of NSW
Located at Centre for Ecotoxicology
c/o University of Technology, Sydney
Westbourne Street, Gore Hill, NSW 2065

ABSTRACT

The risk-based decision scheme devised for deriving site-specific water quality guidelines allows the national trigger values for toxicants to be adjusted to take into account the characteristics of the particular site under study. The scheme provides the opportunity to apply the guidelines in a more realistic manner to take into account the variety of factors that can potentially modify the toxicity of a chemical. It enhances confidence in the use of guidelines at particular sites. It is usually not necessary to proceed through each step in turn at each site, as the approach can be tailored to deal with the significant issues and water quality parameters at that site. For many chemicals, apart from metals, there are limited quantitative data available to enable users to make better estimates of bioavailable concentrations or to adjust trigger values to account for the water quality conditions at the study site. Where only general trends are available, users can only make estimates of increased or decreased risk under the specific conditions. This paper provides some examples, where data are available, of these quantitative relationships for specific chemicals. When data sets were robust, such as for pentachlorophenol, simple overall quantitative adjustments for factors that affect toxicity (in this case, pH) gave as good estimates of changes in the guideline values as more complex manipulations of each data point, followed by recalculation of the guideline value. Background information is given that may assist with qualitative estimates of risk, and other approaches to the decision scheme are discussed, including use of a weight-of-evidence approach to deal with different types of site-specific direct toxicity assessment data. These can assist users of the guidelines to apply the decision scheme as effectively as currently possible, and to focus future research to fill gaps in knowledge.

Key words: Water quality guidelines; application; decision scheme.

INTRODUCTION

The revised water quality guidelines for chemical toxicants (ANZECC & ARMCANZ 2000) list trigger values (TVs) that represent *bioavailable* concentrations of chemicals and these are designed to be applied using a risk-based decision tree framework, described by Chapman (2001). The decision scheme provides practical guidance to water managers on how to apply the trigger values to specific sites in order to account for the local physico-chemical properties of the water. The scheme is intended to account for the effect that these site-specific parameters exert on the overall bioavailability or toxicity of particular toxicants, even if only qualitative estimates can be made at times. Various methodologies can be used to take into account a variety of factors peculiar to the specific site, including comparisons with literature data, theoretical models (eg. Markich *et al.* 2001) or direct toxicity assessments of biological effects (van Dam and Chapman 2001).

Each chemical can be measured initially as a total concentration in an unfiltered sample for comparison with the bioavailable guideline trigger value. Once the chemical type (eg. metal, organic etc.) and the environmental medium (freshwater, marine) are considered, the site-specific decision scheme described in Chapman (2001) begins by considering the level of protection arising from the environmental values of the ecosystem ("Ecosystem conditions", as described by McAlpine and Humphrey 2001). Other factors are then considered, such as the natural background concentration of the chemical, practical analytical quantitation limits, locally important species and the formulation of the chemical. One then considers those water quality parameters that are most likely to affect toxicity, and applies mathematical relationships, where available, between the parameter and the chemical toxicity. Water quality factors that modify the toxicity

of the chemical include hardness, pH, salinity, suspended matter, dissolved organic matter and temperature. For many metals, hardness and speciation are particularly important determinants of toxicity and are described in more detail and illustrated by Markich *et al.* (2001).

The decision scheme also provides guidance on how to examine the effect of simple mixtures of chemicals in ambient waters using theoretical equations (Chapman 2001), but it also provides for the option of undertaking an appropriate suite of direct toxicity assessments (DTA), if at any stage the guidelines are exceeded. DTA can provide the required link between chemical levels and biological effects or establish concentrations that are unlikely to cause harm. There are very few data on the quantitative relationships between water quality parameters and toxicity for most organic toxicants and many inorganic toxicants, apart from metals. In the light of these limitations, this paper provides some background information to help users approach a number of these steps. Further information for each chemical is provided in Chapter 8 of ANZECC & ARMCANZ (2000) to assist managers in approaching site-specific assessments and it is often useful to consult original literature that was used to derive the trigger value.

SELECTING A LEVEL OF PROTECTION FOR DIFFERENT CATEGORIES OF ECOSYSTEM CONDITION

There are three categories of ecosystem condition, incorporating different levels of protection (McAlpine and Humphrey 2001). For the *high conservation/ ecological value systems*, management activities would be expected to ensure that there is no change in biological diversity beyond natural variability (McAlpine and Humphrey 2001), although 99% TVs can be used as default values in these systems until data can be obtained. The trigger values (usually 95% protection) listed in the Guidelines generally apply to *slightly-moderately disturbed* ecosystems. The Guidelines provide the option for water managers to alter the level of protection for *highly disturbed* ecosystems. However, this can only be done if it is scientifically defensible and agreed to by the appropriate bodies. The level of protection can be modified using one of two techniques depending on the method used to derive the chemical's trigger value. If the trigger value was derived by the statistical distribution method (Chapman 2001; Warne 2001) then the percentage of species to be protected can be reduced (eg. from 95% to 90% or 80%). For chemicals whose trigger values were derived using the assessment factor method (Chapman 2001; Warne 2001), reducing the level of protection is more of a problem. In such cases, the most sensitive datum that was used to calculate the TV should be examined to determine whether it is relevant to a local ecosystem. If not, then the trigger value can be recalculated using the most sensitive relevant datum (provided the original acceptance criteria are not violated).

It would be inappropriate to nominate in advance any particular water body as an example of a *highly disturbed* ecosystem, as such decisions are the prerogative of the appropriate key stakeholders, after consultation (McAlpine and Humphrey 2001). If an ecosystem was accepted as *highly disturbed*, a suitable agreed level of protection could be chosen and the trigger values recalculated to this new level. The Guidelines provide a table with 99%, 95%, 90% and 80% levels of protection (at 50% statistical confidence; Warne 2001) for around 82 chemicals in freshwater and 27 in marine water, for which *high* and *moderate reliability* trigger values were calculated using the statistical distribution method. Trigger values that protect an intermediate percentage of species can be calculated if required using the BurrliOZ software (Warne 2001). The trigger values that protect 90% of species are typically 2.7 times larger than those that protect 95% of species but this difference depends on the spread of the original toxicity data. For 26 chemicals in freshwater and 9 in marine water, 99% figures were chosen as default values for *slightly-moderately disturbed* systems, mostly because of their potential to bioaccumulate.

It is important to check that any reduced levels of protection do not approach acute LC50 values. Chemicals for which this may occur are marked in the table of trigger values in ANZECC & ARMCANZ (2000), and the text of the Guidelines gives more detail. The discussion below describes some examples encountered during the derivation process.

TRIGGER VALUES THAT FAILED TO PROTECT AGAINST ACUTE OR CHRONIC TOXICITY

In some cases when deriving the original TVs, the 95% protection level failed to protect against acute or chronic toxicity and the 99% protection level was recommended for *slightly-moderately disturbed* systems instead. This often occurred when there were limited data or when a *high reliability* TV was calculated from acute data but failed to protect against chronic toxicity, or *vice versa*.

Examples include benzene (marine), naphthalene (marine), and in freshwater, 2,4-dinitrotoluene, 1,2,4-trichlorobenzene, 2-chlorophenol and 2,4-dichlorophenol. For benzene in marine waters, a 95% protection figure of 700 µg/L was derived from acute data. The geometric mean of the chronic 40-day NOEC for mortality for the crab *Cancer magister* was 460 µg/L, so the 99% figure of 500 µg/L was recommended for *slightly-moderately disturbed* ecosystems. Even this gives marginal protection against chronic toxicity. For the two chlorophenols, *Daphnia magna* was among the species for which there was inadequate protection from chronic toxicity. Cobalt in freshwater was a case in which 13 acute data points were initially used to derive a 95% protection figure of 92 µg/L and 99% figure of 30 µg/L. However, there were a number of chronic *D. magna* NOEC figures between 2.4 and 20 µg/L, so the assessment factor method was used to give a *low-reliability* TV.

An example of the reverse situation was nickel in marine waters. Chronic data were used to derive a 95% TV of 70 µg/L and a 99% value of 7 µg/L. However low acute figures were reported for *Mysidiopsis bahia* (152 µg/L; USEPA 1986) and the clam *Villorita* sp. (60 µg/L). In addition, low NOEC figures were reported for *M. bahia* (141 µg/L) and the diatom *Nitzschia closterium* (50 µg/L; converted from an EC50). It was predetermined that TVs should not be within 1/3 rd of the lowest acute toxicity value. Hence the 99% figure was chosen for *slightly-moderately disturbed* ecosystems. The situation for cadmium in marine waters was broadly similar, although its tendency to bioaccumulate (Cossa 1988) meant that the 99% figure was chosen in any event.

For endosulfan, neither the limited chronic data nor the acute data provided sufficient protection from acute toxicity at the 95% level. The 95% TV (from chronic data) of 0.2 µg/L was near or above the acute LC50s of several Australian and overseas fish species. Although the 99% figure (0.03 µg/L) was chosen because of its tendency to bioaccumulate, any reduction in the level of protection for endosulfan below 99% should be treated with caution.

There were some chemicals for which even acute data failed to protect from acute toxicity at the 95% level. This is understandable given the application of arbitrary acute-to-chronic conversion factors. For aniline in freshwater, there were only six chronic data points spreading from 4 µg/L to 154 000 µg/L. These would have given *high reliability* figures of 18 µg/L (95%) and 0.15 µg/L (99%) but it was preferred to use the more robust acute data set to derive *moderate reliability* TVs (250 µg/L at 95% and 8 µg/L at 99%). However, several cladocerans had acute toxicity figures between 80 and 250 µg/L, and there were some unverified chronic figures of around 5 µg/L reported for *D. magna*. Hence the 99% figure was recommended for *slightly-moderately disturbed* systems. This was similar for pentachlorophenol (PCP) in freshwater, where a *high reliability* figure (14 µg/L at 99%), from chronic data, did not give sufficient protection from acute toxicity to fish; the lowest acute LC50 was 18 µg/L but there were many figures between 38 and 50 µg/L. Again, the much larger acute data set was used to give *moderate reliability* TVs of 10 µg/L (95%) and 3.6 µg/L (99%). The 99% figure was recommended for *slightly-moderately disturbed* ecosystems both because of the additional protection and because of the potential of PCP to bioaccumulate. TVs from acute data sets failed to protect against acute toxicity at the 95% level for several pesticides in freshwater, and the 99% figures were chosen. These included azinphos methyl, carbofuran, thiram, glyphosate and trifluralin (the latter bioaccumulates). For all of these chemicals, reduction in protection levels should be approached with caution but for most other chemicals, it is possible to reduce the level of protection if a lower ecosystem condition has been agreed to.

NATURAL BACKGROUND LEVELS

Natural background levels of metals may exceed the stated trigger values due to mineralisation from the catchment substrate, as distinct from anthropogenic sources. In highly mineralised catchments, it would be common for those metals that are considered essential for organisms at trace amounts to exceed the trigger values. If the background concentration has been clearly established and it *exceeds* the trigger value, then the 80th percentile of the background concentration is adopted as the site-specific trigger value for ensuing steps. For example, in parts of western Tasmania, high zinc concentrations are found in waters of low hardness and low pH, giving no room to manoeuvre using the hardness algorithms (W. Jones, *pers. comm.* 1998). If the complexing capacity is low, the 80th percentile of the background zinc concentration becomes the guideline value. It is important that any background level is established with confidence. Background levels may vary in different parts of any region and direct toxicity assessment (DTA) may be useful to determine local toxicity effects. However, in such cases it is recommended that indigenous species and individuals are used for the DTA as they may well have different sensitivities to the metals than organisms from areas with lower background concentrations.

Boron levels in seawater are naturally around 4.3 - 5.1 mg/L (Kennish 1989). An absence of marine data prevented calculation of a marine trigger value, but the natural levels of boron in seawater are more than an order of magnitude above the freshwater figure of 370 µg/L. Hence, the 80th percentile of the natural boron level at a marine site would be the default marine guideline value. Similarly, some non-metallic inorganic chemicals such as sulfide, sulfate, ammonia, nitrite and nitrate can occur naturally at elevated levels. Hydrogen sulfide can be released into water both naturally, eg. from anaerobic decay of organic matter, and as a result of human activity. Some organisms may be adapted to high sulfide levels but threshold levels for these are not known.

Few anthropogenic organic chemicals occur naturally at high levels although some, such as DDT residues, are globally distributed and background levels of these may need to be considered.

ANALYTICAL LIMITATIONS

It is recognised that the process for deriving the guideline trigger values for *slight - moderately disturbed* ecosystems (ie. 95% protection values) has produced some figures that are below the practical quantitation limits (PQL; defined in ANZECC & ARMCANZ 2000) of methods in common use in analytical laboratories. PQLs are often around 5 times the method detection limit. The only metals that have TVs below the common PQLs are chromium in freshwater and, in marine waters, cadmium, cobalt and copper. Although methods with lower PQLs are available, they are being used by only a few specialist laboratories. For many pesticides (particularly the organochlorine, organophosphorus, carbamate and pyrethroid insecticides), the concentrations that cause biological effects, and the TVs, may be unmeasurable. If the pesticide can be measured at a site, this indicates that the trigger value has been exceeded and thus a high level of risk is posed to the site. The options are to proceed with management of the site or to proceed to direct toxicity assessment (DTA; van Dam and Chapman 2001). If the PQL is exceeded and it corresponds to a concentration that might cause toxicity, conducting DTA is recommended. This approach has been used to confirm the ecological risk of Sydney Water effluents discharging into the Hawkesbury River, NSW (Bailey *et al.* 2000). Toxicity characterisation techniques were used to establish that organophosphorus pesticides were the main cause of toxicity in these effluents.

Different analytical methods can often measure different chemical entities within a sample. This is a consideration, for example, with cyanide, where the freshwater TV (7 µg/L at 95%) is reported as "unionised HCN, measured as [CN]". The measurement of free-cyanide at carefully-controlled pH with a cyanide-sensitive electrode was considered to be the most reliable measure but many laboratories are only equipped to measure total cyanide, which often will give a higher figure, or weak-acid-digestable (WAD) cyanide, which often will give an intermediate figure. Hence, if the total or WAD cyanide figures are below the trigger value, then one can be confident that the cyanide guideline has been met.

INCORPORATING LOCAL SPECIES

Ideally, when deriving national trigger values it would be preferable solely to use toxicity data for local species tested under local conditions. This was not done in deriving the ANZECC & ARMCANZ (2000) trigger values for toxicants because there were, in general, insufficient toxicity data for Australian and New Zealand species. As more local toxicity data become available it will be possible to recalculate trigger values. This could be done in three ways: local toxicity data could be included with the existing data; data for individual overseas species could be replaced with data for similar species native to the country or region of concern (eg. toxicity data on *Daphnia magna* could be replaced by data on *Ceriodaphnia dubia*); or trigger values could be calculated solely using toxicity data for local species. However, in doing any of the above options it is crucial to maintain the integrity of the trigger values by adhering to the requirements for data quality and quantity (see Warne 2001). It is also important to ensure that a comprehensive overseas dataset is not replaced by an inferior native dataset (ie. that does not contain the same number of taxonomic groups or number of species).

The potential ramifications of recalculating trigger values using only local species toxicity data were examined for phenol and pentachlorophenol, two chemicals for which there is a relative abundance of local toxicity data. The ranges of toxicity values for various taxonomic groups of organisms were similar for both the overseas and local only species. However, the number of taxonomic groups for which there are local toxicity data was considerably less than that used to derive the TVs. In addition, the number of taxonomic groups for which there are local data was not sufficient to be used in the statistical distribution method, and hence only *low reliability* TVs could be calculated. As these two chemicals were amongst those with the most

local toxicity data it indicates that the exclusive use of local toxicity data to derive trigger values is likely only to lead to the derivation of *low reliability* TVs.

The first method of calculating trigger values to include local species toxicity data is therefore recommended. The second and third methods should not be undertaken lightly and are not recommended, except in exceptional circumstances. As more regional and local toxicity data become available, and when there is a greater understanding of relative sensitivity of native species, it may become possible to more confidently use methods two and three.

There could be cases where sensitive and important local species may need special consideration because they are one of the 5% of species that are unprotected. Hickey and Martin (1999) recently reported that the New Zealand freshwater clam *Sphaerium novaezelandiae* was very sensitive to ammonia in 60-day chronic exposures. At pH 7.5 and 20^{0}C, the LC50 and IC50 (juvenile production) figures were 3800 and 800 µg/L respectively, based on total ammonia (NH_3-N). At this pH, the TV (95% protection) for total NH_3-N is 1650 µg/L, which may provide marginal protection for this species. In waters where this or related species are important, it may be necessary to adopt a lower site-specific value for ammonia but because of the very large data set for ammonia, it was not considered appropriate to choose a 99% trigger value for *slightly-moderately disturbed* ecosystems.

CHEMICAL FORMULATION

The vast majority of toxicity tests have been conducted using technical grade chemicals, whereas many types of chemicals are usually introduced to the environment as chemical formulations. Formulations contain a number of additives some of which have been shown to change the toxicity of the active ingredient (eg. Solon *et al.* 1969; Folmar *et al.* 1979; Beusen and Neven 1989). Due to the potential for the chemical and the additives to interact, concern has been raised over whether the toxicity tests conducted using the technical material are relevant. A large review of the relative toxicity of technical and formulation forms of pesticides has been conducted by Schmuck *et al.* (1994). They found that pesticide formulations were not more toxic than the technical grade 75, 65 and 75% of the time for green algae, cladocerans and fish respectively. Also they found that at least 98% of all formulations were no more than 10 times more toxic than the technical material to those organisms. Where the relative toxicity of formulations and technical material is known, appropriate adjustments to the TVs can be made. Some examples of the differences between the toxicity of technical material and formulations are given below.

Recent concerns about the higher toxicity of a glyphosate formulation (Roundup®), compared to the parent compound, led to restrictions on its use near Australian waterways (NRA 1996). The trigger value derived for technical grade glyphosate (370 µg/L for 99%; as reported in the Guidelines) should be divided by 40 if the common Roundup® formulation is used (ie. to give 9 µg/L). A new formulation called Glyphosate Biactive® with a low toxicity surfactant has been developed for use near water. Unpublished data indicate, at this stage, that the lowest LC50 for the Biactive® formulation is >300 mg/L compared to 3 mg/L for the technical grade glyphosate. However, the relative toxicity needs to be assessed in the peer-reviewed literature before a correction factor can be derived and a trigger value for Biactive® calculated.

Another example of formulations modifying toxicity is for two commercial formulations of eight xanthates used for mineral flotation in the mining industry. Their toxicity (for the same xanthate type) varied by up to one order of magnitude (NICNAS 1995). This difference emphasises that care will be required in site-specific interpretation of xanthate data.

Formulations can also be affected by environmental conditions. For example, the toxicity of Roundup® increased by a factor of between 2 and 6 (depending on test species and duration) as pH increased from 6.5 to 7.5, but did not change further up to pH 9.6 (Folmar *et al.* 1979). Care also needs to be taken when considering the toxicity of different 2,4-D formulations at different ambient pH levels. For instance, the acid, butyl ester and diethyl amine salt were less toxic to *Pimephales promelas* at pH 8.5 than at pH 6.5 by a factor of about 2, whereas the dodecyl/tetradodecyl amine salt was 3.5 times more toxic at the higher pH (Johnson and Finley 1980). At this stage, these considerations can only be noted as factors that may increase or decrease the risk at the specific site.

Other factors that limit the relevance of laboratory-based experiments to the environment are the differential transport of the additives and the active ingredient, so that many of the additives would not reach the water, and/or would degrade rapidly (Schmuck *et al.* 1994). The above examples highlight that the results of laboratory-based toxicity tests with formulations and technical material should be considered carefully before extrapolating to the environment.

CHEMICAL OR WATER QUALITY FACTORS THAT MODIFY TOXICITY

Adsorption/desorption on suspended matter

The interactions of toxicants with suspended material are complex and vary with concentration of the chemical, concentration of the suspended material and properties of the chemical. At the current stage of knowledge, it is not possible to make quantitative adjustments of TVs to account for suspended matter. The following information may assist users in making qualitative judgements on whether risk is increased or decreased at the site, or whether there is value in proceeding directly to DTA. Some metals adsorb strongly to clay particles, thereby reducing their bioavailability. Filtering of non-acidified samples (eg. 0.45 μm) to estimate the degree of bioavailability is discussed, along with effects of hardness on the metal toxicity, by Markich *et al.* (2001).

Caution needs to be exercised whenever considering adsorption of chemicals to suspended matter, as this process is dynamic and reversible, and dependent both on the nature of the chemical and the binding sites on the particulates. The bioavailability of contaminants attached to particles is poorly understood. Exposure routes such as adsorption across biological membranes such as fish gills or ingestion will be important here. Also, the issue of saturation of binding sites for a particular chemical on suspended particles is not clearly understood and can vary from site to site due to changes in soil chemistry within a catchment.

The binding of organic chemicals to suspended matter is predominantly controlled by the logarithm of the octanol-water partition coefficient of the chemical (log Kow) and the organic carbon content of the suspended matter. Typically chemicals with log Kow values greater than 3 are considered to have the potential to bind to suspended matter sufficiently to reduce their bioavailability.

The effect of suspended matter on the bioavailability of organic chemicals is illustrated by research on endosulfan and the pyrethroid deltamethrin. Sunderam (1990) reported that the high toxicity of endosulfan to rainbowfish *Melanotaenia duboulayi* in clean laboratory water was reduced by addition of kaolinite and bentonite clays. However, Sunderam *et al.* (1992) found that the acute toxicities of endosulfan to native species in turbid Mehi River water were not significantly different from those of the animals tested in the filtered Sydney mains water. Patra (1999) similarly, found that the endosulfan LC50 values for silver perch *(Bidyanus bidyanus)* in mesocosms (with suspended solids and sediments) were similar to those conducted using laboratory water (with no suspended solids or sediments). Leigh *et al.* (1997) found that suspended sediment, modelled on the particulate size characteristics and typical loads of water from the Namoi River, NSW, did not ameliorate the acute 24-h toxicity of endosulfan to *M. duboulayi* under static conditions. However, a very high suspended sediment load of 52 mg/L (ie. approximately two orders of magnitude greater than those typically found in rivers of the study area; Gordon 2001) caused a 2.2 fold reduction in toxicity. If further assessment of endosulfan risk is to be undertaken, it would be necessary to assess whether endosulfan at low concentrations (eg. around the 99% TV, 0.03 μg/L) is significantly bound to suspended sediment at turbidity levels typical of the cotton growing areas.

Pyrethroids such as deltamethrin and esfenvalerate have very high log Kow (often ≥ 6), which suggests that they would be strongly bound to sediment or suspended matter and would be transient in the water column. Thomas (2001) examined the effect of suspended matter and bottom sediment on the toxicity of deltamethrin to the freshwater shrimp *Paratya australiensis* under field conditions. She found that slightly turbid water from the Namoi River, NSW, only provided slight amelioration (1.5 fold decrease) of the acute toxicity of deltamethrin, whereas the presence of sediment reduced the toxicity six fold.

The above examples indicate that more research is required in this area, and that at present there is limited scope for changing pesticide TVs due to high suspended matter. DTA may assist with decisions in areas where turbid waters may be thought to affect toxicity.

Incorporating dissolved organic matter

Most of the influences that govern the interrelationships between chemicals and suspended material also apply to dissolved organic matter (DOM) or total organic carbon (TOC). Again, metals can strongly adsorb to organic matter and become less available. In many cases, it may not be necessary to know the nature of the DOM if the overall toxicity amelioration can be determined using direct toxicity assessment. For example, the toxicity of filtered (0.45 μm) water from the Ok Tedi/Fly River system in PNG was determined using the sensitive unicellular algal growth inhibition bioassay with *Chlorella protothecoides* (Stauber 1995), as a tool to check if the copper was in the bioavailable form. Dissolved copper concentrations were between 0.5 and 12.6 μg/L and decreased with increasing distance from the Ok Tedi mine. However, none of the samples were toxic to the

algae, even though growth of this species was inhibited by copper at concentrations as low as 3 µg/L in laboratory medium. This of course is not a full DTA procedure (which should involve a suite of at least 3 species that are taxonomically-representative of different trophic groups), but it does suggest that copper was complexed or bound and unavailable for uptake into the algal cells. This was improved by chemical measurement of the copper-complexing capacity of the DOM in the water.

Similarly, in Macquarie Harbour in Tasmania, dissolved copper concentrations from mining operations of between 10 and 42 µg/L were not significantly toxic to three species: the microalga, *Nitzschia closterium* (72-h growth); the amphipod *Allorchestes compressa* (27-d growth and survival); or the fish *Rhombosolea tapirina* (14-d survival, histopathology, copper accumulation and osmoregulatory disturbance). In contrast, significant toxicity occurred at or below 5 µg/L for algae and amphipods in laboratory tests with ionic copper. Hence this DTA approach indicated that the copper in the Macquarie Harbour waters was not bioavailable to the species tested (Stauber *et al.* 1996). In summary, given the current state of knowledge, use of DTA procedures in conjunction with chemical analyses appears to offer the best approach to dealing with the effects of organic matter on the chemical toxicity. Van Dam and Chapman (2001) describe how to incorporate DTA results into quantitative site-specific guideline assessments.

Incorporating pH effects

The acidity of water affects the speciation and bioavailability of many heavy metal ions in solution and hence their toxicity. At high pH values, many metals will precipitate out as metal hydroxides or other salts thus reducing their toxicity. However, this is really only relevant to freshwaters as seawater is well buffered and hence its pH does not vary greatly. For this reason toxicity data were only used to calculate freshwater trigger values if the pH at which the tests were conducted was between 6.5 and 9 (Warne 2001).

Some metals such as aluminium are more toxic at low and high pH than at neutral pH. In order to reflect this, trigger values covering different pH ranges should be calculated, but there were only sufficient data for aluminium at different pH levels to calculate TVs for pH >6.5 and a low reliability TV for pH <6.5. Separate freshwater trigger values (ie. PC95%) were developed in the Guidelines (ANZECC & ARMCANZ 2000) for pH < 6.5 (0.8 µg/L) and for pH > 6.5 (55 µg/L).

The toxicities of some organic chemicals, eg. weak acids or bases, are also affected by pH. This is because pH affects the extent of ionisation (ie. ratio of unionised and ionised forms) of these chemicals. As a general rule only a small proportion of the ionic form of a chemical crosses lipid membranes whereas a much greater proportion of the unionised form crosses membranes. Thus, the ionic state can have a major effect on the bioavailability and toxicity of chemicals. The relationship between pH and the extent of ionisation is described by the Henderson-Hasselbalch equation (Connell *et al.* 1997):

$$pH = pK_a + \log [base]/[acid] \quad (1)$$

where pH is the negative logarithm of the hydrogen ion concentration (-log[H^+]) and pK_a is the negative logarithm of the acid ionisation constant (-log K_a). Any change in pH will change the extent of ionisation. A variation of one pH unit will cause a ten-fold change in the ratio of ionised to unionised forms. This will, in turn, have significant effects on the ability of chemicals to be transferred across lipid membranes.

Phenols are one group of organic chemicals that are affected by changes in pH. For pentachlorophenol (PCP), the USEPA (1986) has developed algorithms to derive their 1-h and 4-d maximum average criteria that vary according to pH. The 4-d algorithm is e[1.005(pH-5.29]. There are at least two different methods of deriving pH-modified trigger values. The most accurate method would be to first adjust each toxicity data point used to calculate the TV, to the pH appropriate for the site using equations such as the USEPA equation, then recalculate the TV. However, a simpler, less calculation-intensive alternative would be to determine the mean pH of the data used to calculate the guideline TV, then adjust the TV to the appropriate pH using the USEPA equation. We compared the resulting TVs obtained by using the two methods on the data used to derive the PCP TV. We used the 4-d USEPA equation (most applicable to protect from chronic exposure) to calculate TVs for PCP at different pH levels using both of these approaches. If the simpler approach gave a similar pH-modified TV to the more complex method, then this gives users greater confidence in using broad scale approaches for other adjustments, where quantitative relationships are available.

For the first method the toxicity of each toxicity value was adjusted to the appropriate pH, then the geometric mean was calculated for each species before entering the pH-modified data into the BurrliOz program and the trigger value calculated (column A, Table 1). Trigger values modified for pH were calculated for pH values of 6, 6.5, 7.2, 7.8, 8.4, and 9. In effect, any LC50 value for which there was no pH reading was deleted.

For the second method the arithmetic mean of pH for all the toxicity data was calculated to be 7.57. It was assumed that the original TV (99% protection level; 3.6

µg/L) applied to this pH. The USEPA equation was then used to calculate TVs at the same pHs as the first method (column B, Table 1).

The pH-modified trigger values obtained using the two methods were very similar (compare columns A and B, Table 1). However, it is interesting to note that the TVs calculated using the simpler method were always slightly lower (ie. more protective) than those calculated by the more complex method. The calculations suggest that it may be possible to use simple adjustment methods rather than more complex methods. However, as it is based on comparing results for just one chemical, this is far from conclusive.

Table 1. *Trigger values for PCP (µg/L; 99% protection) at different pH levels – calculated from different arrangements of data using the USEPA (1986) equation (recommended figures in bold).*

pH	A	B
6.0	0.9	**0.7**
6.5	1.6	**1.2**
7.2	3.3	**2.5**
7.57	4.8	**3.6**
7.8	5.9	**4.5**
8.4	11.3	**8.4**
9.0	19.9	**15**

Columns A & B full descriptions in text
A = USEPA equation applied to 99% TV of 3.6 µg/L at overall pH of 7.57 (arithmetic mean).
B = pH for each line entry in the Guidelines database noted and LC50/EC50 recalculated adjusted using USEPA equation, species geometric mean of toxicity calculated, before BurrliOz.

Table 2. *Factors to adjust phenol toxicity for pH and suggested 95% protection trigger values for phenol at different pH levels (preferred precautionary factors are in bold).*

	Factors		Phenol TV
pH	*N. notopteris*	*H. fossilis*	µg/L
4.6	**0.22**	0.34	70
5.5	**0.7***	0.75*	200
6.0	**0.9**	0.87	270
6.8	0.95*	0.95*	285
7.3	**1**	**1**	300
7.6	1.05*	**1.05***	320
8.0	1.3*	**1.15***	350
8.8	2.3	**1.4**	420

* By graphical interpolation.

PCP is the only organic chemical for which a quantitative pH-toxicity algorithm is available but there are some indications of changes in toxicity of other chemicals with pH. Several authors observed an increase in the toxicity of phenol to fish with decreasing pH, but the most useful pH-phenol toxicity relationships were reported by Dalela *et al.* (1980) for three fish species, *Colisa fasciata*, *Heteropneustes fossilis* and *Notopteris notopteris*. All showed similar relationships with steep pH-LC50 curves for phenol at pH <6 and >7.5, and shallower in between 6 and 7.5. The largest differences were for *N. notopteris*; if the LC50 at pH 7.3 was taken as a factor of 1, the figures at pH 4.6, 6.0 and 8.8 were 0.22, 0.9 and 2.3 times that at pH 7.3 (Table 2). For comparison, the equivalent factors for *H. fossilis* were 0.34, 0.87 and 1.4. The TV (95% protection) for phenol was 320 µg/L and the mean of the pH readings was 7.6. From a plot of the *N. notopteris* figures, the TV at pH 7.3 would be 0.95 times that at pH 7.6, ie. 300 µg/L. On this basis, the TV at pH 4.6 would be 70 µg/L, at pH 6 it would be 270 µg/L and at pH 8.8 it would be 690 µg/L. To be more precautionary at higher pH values, the *H. fossilis* factor at pH 8.8 is recommended, to give a TV of 420 µg/L (Table 2).

The toxicity of other organic chemicals can also be affected by pH. Toxicity of chlorpyrifos to cutthroat trout *Salmo clarki* increased by 3 times when the pH was increased from 7.5 to 9.0, but pH (6.4 - 9) did not alter the toxicity of either fenitrothion or diuron (Johnson and Finley 1980). Changes in pH can either accelerate degradation of some organic chemicals or retard it. The OP pesticide profenofos is many orders of magnitude more stable in acidic water (Tomlin 2000): the calculated 50% hydrolysis time for profenofos at 20°C was 93d at pH 5, around 15d at pH 7 and 0.25d at pH 9. This may not affect the guideline value but will have some bearing on the exposure side of any risk assessment. The field rates of degradation in the alkaline waters of the cotton growing areas may actually be much slower than this (Kumar and Chapman 2001).

The toxicity of several inorganic ions is affected by pH. For instance, the toxicity of ammonia is governed by the equilibrium between free ammonia and the ammonium ion (NH_4^+), which is in turn largely a function of both pH and temperature. The Guidelines (ANZECC & ARMCANZ 2000) provide a table of total ammonia (NH_3-N) trigger values at different pH levels: the freshwater TV at pH 6 is 2.6 mg/L; at pH 7 it is 2.2 mg/L; at pH 8 it is 0.9 mg/L; and at pH 9 it is 0.18 mg/L. Equilibrium equations and tables in the Guidelines allow users to calculate un-ionised ammonia levels for different pH and temperatures. Analogous tables are also given for hydrogen cyanide and hydrogen sulfide.

Incorporating temperature

High water temperatures in Australia have the potential to influence the toxic responses of aquatic organisms, and should be considered wherever applicable and possible. Quantitative relationships between temperature and toxicity have been derived for very few chemicals, but those that have, can be used to adjust TVs for the temperature at specific sites. Where the relationships differ for different species, it is recommended that users choose the most precautionary relationship when making any adjustments. For most chemicals, it is only possible, at this stage, to make qualitative estimates of increased or decreased risk at specific sites.

Many organic chemicals exhibit between a two and four fold increase or decrease in toxicity for each 10°C rise in temperature (Mayer and Ellersieck 1986). However, literature on the effect of temperature of some chemicals is also conflicting, probably due in part to competing processes of increased rate of intake of chemical and rate of toxic action versus rate of excretion at higher temperatures, as well as other factors such as increased degradation.

The relationships between temperature and toxicity were assessed for a number of Australian freshwater species by Johnston *et al.* (1990) and Patra (1999). The relative changes in toxicity of three chemicals to different species from these studies are presented in Table 3, along with results from other studies from the database (Sunderam *et al.* 2000) in ANZECC & ARMCANZ (2000) in which toxicity was measured at different temperature levels in the same study. The relationships were not necessarily the same for fish and invertebrates but it is considered valuable to adopt a precautionary approach and make worst-case assessments. For phenol, data from 155 species contributed to the calculation of the TV, and 122 had concurrent temperature measurements; the geometric mean (gm) for temperatures was 19°C. Taking worst-case approaches (factors from columns 4 and 6 of Table 3), the TV of 320 µg/L (95% protection) applying at 19°C would convert to 260 µg/L at 15°C, 230 µg/L at 25°C, 170 µg/L at 30°C and 160 µg/L at 35°C. The 99% TV for PCP (3.6 µg/L) was calculated from data with a gm for temperature of 19°C. The approximate TVs at 15°C, 25°C, 30°C and 35°C were calculated to be 2.0, 4.5, 3.8 and 1.4 µg/L respectively, based on *M. duboulayi* figures.

Table 3. *Some temperature – acute toxicity relationships for phenol, pentachlorophenol (PCP), endosulfan, and chlorpyrifos: factors at different temperatures. Factors were calculated by dividing the toxicity figure (acute LC50, except where noted) at the listed temperature by the toxicity figure at 20°C (hence the factor is "1" at 20°C). Preferred (precautionary) adjustments for each chemical are in bold.*

	Phenol						PCP		Endosulfan		Chlorpyrifos	
	M.d[1]	*N.n*[2]	*O.l*[3]	*B.b*[4]	*C.d*[1]	*C.d*[4]	*M.d*[1]	*C.d*[4]	*B.b*[4,5]	*C.d*[4,6]	*B.b*[4]	*C.d*[4,6]
10°	-	-	2.5	-	-	-	-	-	-	-	-	-
15°	0.8	-	-	**0.8**	2.1	1.8	**0.55**	1	1.4	**0.4**	**2.1**	**3**
16°	-	1	-	-	-	-	-	-	-	-	-	-
20°	-	-	**1**	**1**	1	**1**	**1**	1	1	**1**	**1**	**1**
23°	-	1	-	-	-	-	-	-	-	-	-	-
25°	1	-	-	1.3	0.46	**0.73**	**1.25**	1.4	1.1	**0.1**	0.8	**0.1**
30°	-	-	0.75	1.3	0.26	**0.54**	**1.05**	1.4	0.7	**0.05**	0.65	**0.1**
35°	1.2	-	-	**0.5**	-	-	**0.4**	-	0.75	-	0.55	-
36°	-	0.5	-	-	-	-	-	-	-	-	-	-

* from graphical interpolation.

M.d = Melanotaenia duboulayi; *N.n = Notopteris notopteris*; *O.l = Oryzias latipes*; *B.b. = Bidyanus bidyanus*; *C.d. = Ceriodaphnia dubia*; 1 = Johnston *et al.* (1990) (nominal); 2 = Gupta *et al.* (1983); 3 = Tsuji *et al.* (1986); 4 = Patra (1999) (measured, except for chlorpyrifos); 5 = 24h LC50 (otherwise 48 or 96h); 6 from chronic LOEC data (measured).

For endosulfan, chronic freshwater toxicity data were used to calculate the 99% TV of 0.03 µg/L. There were only 10 individual chronic figures and only 5 with recorded temperatures – the geometric mean of temperature was 22°C. Taking a precautionary approach, from the chronic *C. dubia* figures, the TV at 15°C would be 0.02 µg/L, at 25°C it would be 0.003 µg/L and at 30°C it would be 0.002 µg/L (the latter two figures are well below the analytical PQL). Given the limited data, the low readings at 25°C and 30°C suggest that endosulfan at higher water temperatures may be more detrimental to invertebrates. This is supported by similar trends with acute *C. dubia* data from Patra (1999). For chlorpyrifos, there were 46 species with acute data (32 with associated temperature readings) but there were only 9 species with chronic data and very few concurrent temperature readings. The chronic data had been used to calculate the TV (95%) of 0.01 µg/L, and the geometric mean of temperature of 19°C was assumed to apply to the TV. Again, taking a precautionary approach, the TV at 15°C would be 0.03 µg/L, and at both 25°C and 30°C it would be 0.001 µg/L. As all these figures are below the current analytical PQL, the figures can do no more than suggest a greater risk at higher temperatures.

Increased temperature generally increased toxicity of a number of other organophosphorus pesticides to freshwater organisms. A twofold increase occurred in toxicity of azinphos-methyl to *O. mykiss* between 2°C and 18°C and for bluegills *P. promelas* between 12°C and 22°C. A much larger increase in toxicity, 17 times, occurred for yellow perch *Perca flavescens* between 7°C and 22°C (Johnson and Finley 1980). The toxicity of diazinon was significantly increased at higher temperatures. The 48-h LC50 to *Aplocheilus latipes* decreased from 24 000 µg/L at 10°C, to 11 000 µg/L at 20°C and 600 µg/L at 30°C, an overall increase of 40 fold (Tsuji *et al.* 1986). However, temperature (7 to 17°C) did not alter toxicity of fenitrothion (Johnson and Finley 1980). Toxicities of herbicides 2,4-D and glyphosate to various species doubled for every 10°C increase in temperature (Johnson and Finley 1980) but there was no change in toxicity of diuron to trout *O. mykiss* (2 to 8°C) and *P. promelas* (7 to 24°C). In summary, it is only for phenol and PCP that quantitative adjustment of the TV for temperature is currently possible, while for other chemicals, such as endosulfan and chlorpyrifos, higher temperatures increase the risk of toxicity effects. In the absence of data, the TV should be at least halved for a 10°C rise in temperature from that used in tests to derive the toxicity data used to generate the trigger values.

There is little information regarding the influence of temperature on the toxicity of chemicals to marine organisms. Despite this, one might expect temperature to have similar effects.

Effects of water salinity

Salinity can affect the toxicity of metal, inorganic and organic chemicals and this has been reviewed by Hall and Anderson (1995). Such changes are only relevant for fresh and brackish waters where the salinity often varies. These interactions or quantitative relationships are potentially very important for Australia given the current trend of increasing salinity in both ground and surface waters (DLWC 2000; AGSO 2001). Generally an increase in salinity would be expected to decrease the solubility, availability and hence the toxicity of metals (Hall and Anderson 1995), although Gorrie *et al.* (in press) found that the bioavailability of copper and zinc to *Chironomus maddeni* in sediments did not conform to this general observation. The changes of metal bioavailability as a function of salinity is a complex area involving changes in metal speciation, and is discussed in more detail by Markich *et al.* (2001). Hall and Anderson (1995) found no consistent trend for the effect of salinity on the toxicity of organic and inorganic chemicals, except for organophosphorus pesticides, the toxicity of which increased with increased salinity. As far as the authors of the present study are aware, no quantitative relationships have been developed relating salinity and toxicity. Therefore at present, there are few opportunities to adjust TVs for this factor. Readers are recommended to read Hall and Anderson (1995) for details of how salinity affects the toxicity of other chemicals. However, some specific examples of how salinity affects toxicity of other chemicals are given in the following text.

Johnston *et al.* (1990) found that increased salinity up to 5000 mg/L NaCl did not affect the toxicity of phenol to the Australian rainbowfish *M. duboulayi* at 25°C. However, phenol was 2-2.5 times more toxic to the waterflea *C. dubia* at 30 and 100 mg NaCl/L (48-h) than at 1000 and 2000 mg NaCl/L. In contrast, the 96-h LC50 for PCP to *M. duboulayi* at 25°C decreased from 1.5 mg/L at low salinity (30 mg/L NaCl) to 0.28 mg/L at 5000 mg/L NaCI. However, there was no change in toxicity of PCP to *C. dubia* at salinities between 30 and 2000 mg/L (Johnston *et al.* 1990).

Hall *et al.* (1995) demonstrated that the chronic toxicity of atrazine to the estuarine copepod *Eurytemora affinis* decreased as salinity increased from 5 to 15ppt with EC50 values of 14.5 and 20.9 mg/L respectively. This may have a slight bearing on the interpretation of atrazine risk in estuarine environments.

Effects of water hardness

Markich *et al.* (2001) details the effects of hardness on metals. There are few data on the effect of hardness on organic chemicals, and hence less opportunities to adjust their TVs for hardness. However, where there are quantitative data given, it would be reasonable to adjust the TVs accordingly for hardness, taking the most precautionary approach. Some examples of the effects of water hardness on the toxicity of organic chemicals are given below. The toxicity of glyphosate in soft water (around 5-10 mg/L $CaCO_3$) was approximately half that in water of intermediate hardness (around 50 mg/L $CaCO_3$) (Wan *et al.* 1989). In contrast, toxicities of parathion, fenitrothion and other organophosphorus pesticides, and of diuron and 2,4-D were little affected by changes in hardness (44 to 300 mg/L $CaCO_3$) (Johnson and Finley 1980).

Literature on the effect of hardness on phenol toxicity is contradictory. Rainbow trout *O. mykiss*, carp *C. carpio* and mosquitofish *Gambusia affinis* were less sensitive to phenol in hard water than in soft water but there was no difference for *P. promelas* (Johnston *et al.* 1990). In contrast, Birge *et al.* (1979; cited in Johnston *et al.* 1990) found that the toxicities of phenol to early life-stages of *O. mykiss* and *Carassius auratus* increased by factors of 7 and 3 respectively, as hardness increased from 50 to 200 mg/L.

Incorporating transient exposure and rapid degradation of the chemical

The Guideline trigger values are derived using data from chronic or acute toxicity tests where the organisms are exposed to a constant concentration of the toxicant for the duration of the test. When dealing with constant effluents, relatively persistent chemicals or elevated levels from diffuse sources of pollution, the trigger values in the Guidelines (ANZECC & ARMCANZ 2000) provide a best estimate of no-effect levels. It is known that, as the duration of exposure increases, the concentration needed to cause a given toxic effect decreases. Hence, for some transient (<24h) exposures, the TVs may be overprotective. However, the current state of knowledge on this issue does not allow users to make any adjustments due to transient exposures. The discussion below highlights some examples where transient exposures can cause sustained toxic effects and outlines international progress in dealing with this issue.

For some chemicals, such as chlorine, their transient occurrence in the environment (except in continuous discharges) was taken into account to some extent by including 24-h toxicity data in the derivation procedure. Unfortunately, there was little international guidance on how to account for transient peaks when deriving or applying guidelines. The USEPA (1986) have included averaging periods within their guidelines, which for acute criteria are 1-hour averages and for chronic criteria are 4-day averages, both not to be exceeded more than once every three years on average. The concepts of kinetic modelling of exposure and species recovery are being considered by USEPA (Delos 1994) but no further developments have been published (C Delos, USEPA, *pers. comm.*, 2000).

Responses of organisms to pulse exposures are not easily predicted from normal acute and chronic toxicity data. Australian laboratory data indicate that even very short-term pulse exposures (< 1.5 h) of rainbowfish *M. fluviatilis* to deltamethrin at concentrations as low as 0.09 µg/L (Holdway *et al.* 1994), can result in delayed mortality. Similarly, the USEPA (1984) reported delayed toxicity to rainbow trout after 64 - 139 days following a 6-h exposure to 0.1 µg/L of TCDD (dioxin). Abdullah *et al.* (1994) demonstrated that repeated 24-h exposures of the shrimp *Paratya australiensis* to profenofos (0.1 µg/L) with 7 days recovery in between resulted in greater inhibition of acetylcholinesterase activity after the second and third doses, despite almost full recovery of AChE levels in between. In contrast, Kallander *et al.* (1997) found that episodic exposure to carbamate and OP pesticides caused less toxicity to *Chironomus riparius* than continuous exposure at the same concentrations provided there was at least 6 hours between doses.

The vast majority of monitoring or sampling carried out use grab samples that provide an instantaneous estimate of the concentration. Due to the relative infrequency of the collection of grab samples they are highly unlikely to contain chemicals that occur as pulses of short duration. Continuous sampling can be used to overcome this limitation, however, such procedures are not suitable for chemicals which can not be measured instantaneously. An alternative is the use of passive samplers, such as diffusive gradients in thin films for metals (eg. Zhang and Davison 1995) and semi-permeable membrane devices for organic chemicals (Muschal 1999), which provide a time-weighted average concentration for the sampling period. Because they continually absorb pollutants from the ambient water, these techniques have been able to detect several pesticides in the north-western rivers of NSW that were not detected in fortnightly spot water sampling (Muschal 1999). There are problems relating the concentration in the samplers back to the concentration in water for chemicals that vary considerably over time. However, Leonard *et al.* (2001) reported a quantitative relationship between the aqueous and sampler concentration for endosulfan under laboratory flow-through conditions, which may

help resolve this issue. In any event, transience in water may not necessarily mean transience in sediments, and sediment guidelines may need to be assessed separately.

For OP pesticides that contain sulfur groupings, toxicity can be increased by oxidation to the more toxic oxon analogue. Degradation in water over 1 to 3 weeks increased azinphos-methyl toxicity between 1.3 and 2 times. In contrast, degradation of fenitrothion over 3 weeks did not alter its toxicity (Johnson and Finley 1980). Similarly for the herbicide diuron, ageing of test solutions for 7 days, possibly allowing oxidation of the C-S bond, caused increased toxicity in the crustacean *Gammarus sp.* but not in fish (Johnson and Finley 1980). This type of information may assist understanding of the exposure side of any risk assessment but does not assist greatly with assessing effects of transient exposure and in translating such effects into guideline trigger values. This is an important area for future research.

Incorporating toxicant mixtures

The trigger values in the Guidelines (ANZECC & ARMCANZ 2000) are chemical-specific and hence do not take into account that other compounds may be present and also exerting toxic effects. The decision scheme in the ANZECC & ARMCANZ (2000) Guidelines does, however, provide a mechanism to consider chemical mixtures. If the mixture is a simple one, comprising generally just a few chemicals with same mode of action (ie. where additive toxicity is expected), whether or not a mixture provides the intended level of protection could be determined using the formula (see Chapman 2001):

$$TTM = \sum(Ci / TVi) \qquad (2)$$

where *TTM* is the total toxicity of the mixture, *Ci* is the concentration of the 'i'th component in the mixture and *TVi* is the guideline for that component. If TTM exceeds 1, the mixture has failed to provide sufficient protection. Further, if the aqueous concentration of any chemical in the mixture exceeds its guideline TV, then the guidelines are automatically exceeded.

This equation can apply to simple mixtures such as benzene, toluene, ethylbenzene and xylenes, collectively known as BTEX, and commonly encountered in contaminated petroleum sites. The toxicity of these components is additive but their TVs comprise a mixture of *moderate* and *low reliability* (l.r.) values: benzene 950 µg/L; toluene 180 µg/L (l.r.); ethylbenzene 80 µg/L (l.r.); *o*-xylene 350 µg/L; *m*-xylene 50 µg/L (l.r.); *p*-xylene 200 µg/L. If hypothetical aqueous concentrations (C) of these chemicals are 300, 100, 50, 200, 20 and 100 µg/L respectively, each chemical is below its TV. The values of the fraction (C/TV) would be 0.32, 056, 0.62, 0.57, 0.27 and 0.5 respectively but the sum of these (ie. the TTM) is 2.84, indicating that the mixture poses a high risk of adverse effects at this hypothetical site.

When the mixture is complex (ie. > 5 components) and/or contains chemicals with different modes of action then the previous TTM equation can not be used. In such cases the literature should be searched for data on the toxicity of the mixture or direct toxicity assessment (DTA) should be used. This is emphasised by recent Australian studies with two OP pesticides and endosulfan (Woods and Kumar 2001; Woods *et al.* 2001), which showed complex synergistic or antagonistic toxicity interactions, even between the two OPs, which could not be easily predicted from general considerations of chemical structure.

Incorporating direct toxicity assessment (DTA)

DTA of the effluent or ambient water is the best method to take into account the toxicity of mixtures, as this determines the total toxicity of the mixture, irrespective of the types of interactions between the components that occur. DTA is an approach that is complementary to chemical-specific guidelines and is adopted in many OECD countries (van Dam and Chapman 2001) to quantify the toxicity of wastewater and establish discharge criteria. Methods and protocols are currently available for testing numerous Australian species (van Dam and Chapman 2001).

DTA is recommended in the Guidelines (ANZECC & ARMCANZ 2000) as an alternative approach for deriving site-specific guidelines in substantially natural and highly modified ecosystems. With this method, organisms local to the area are used as test species, and the local receiving water (eg. from a clean reference site) is used as the control and dilution water. DTA can also include *in situ* testing, where organisms are exposed to the test water in the actual environment. For example, the response of fish in cages may be quantitatively compared with equivalent caged fish in a clean reference area to derive EC50, LC50, NOEC and LOEC figures. Van Dam and Chapman (2001) discuss DTA in detail, but it is important that the duration of exposure, and test endpoints are realistic representations of the exposed environment. It is also important to apply a suite of toxicity tests to represent different trophic levels and also to satisfy the minimum data requirements of either the statistical distribution or assessment factor methods (Warne 2001). Van Dam and Chapman (2001) presented a number of case studies of the application of DTA while this paper will present another example for endosulfan.

A site-specific trigger value was derived using site-specific DTA data on the pesticide endosulfan from inland-river mesocosms near the Namoi River and from other related studies with local waters (Table 4) (Hose 2000; Leonard *et al.* 1999, 2001; Brooks 1998; S Wilson, Aust. Catholic Uni. *pers. comm.*). The mesocosms, containing local fish and invertebrates, were dosed on three occasions with endosulfan under different conditions and the responses of these species were recorded and calculated as measured acute LC50, LOEC, and NOEC values. Three species of insect were tested and as they had different functions in the local ecosystem they were considered to belong to different taxonomic groups when site-specific calculations were conducted using the statistical distribution method (Warne 2001). Geometric means of measured acute NOECs for the tested species were: 1.1 µg/L for *Jappa kutera;* 3.7 µg/L for *Atalophlebia australis*; 0.35 µg/L for *Cheumatopsyche* sp; 0.5 µg/L for the crustacean *Paratya australiensis*; and 0.34 µg/L for juvenile *Macquaria ambigua*. Additional acute LC50 data for four local fish species from Sunderam *et al.* (1992) were converted to acute NOEC values (Table 4) using the method outlined in Warne (2001).

The NOEC data were used in the program BurrliOz (Campbell *et al.* 2000) to derive acute protection figures (99%; at 50% confidence) of 0.005 µg/L, compared to 0.03 µg/L for the original 99% TV. To derive chronic site-specific values, the acute protection figures were multiplied by an appropriate acute-to-chronic ratio (ACR). The acute-NOEC/chronic-NOEC ratio is 2.98. The resultant 99% protection figure of 0.002 µg/L is well below the Guideline TV for endosulfan of 0.03 µg/L. The results may apply to inland streams and rivers with similar dominant taxa to those tested but are well below the analytical PQL. However, it is important to note that the range of species tested is relatively limited and the endpoints were acute, requiring a further acute-chronic conversion. Hence, it may currently be preferable to remain with the national guideline. The results do, however, suggest that the national guidelines may not be overly conservative. Nevertheless, the process is a useful illustration of the application of one of the most comprehensive site-specific toxicity data sets available for a pesticide in Australia to derive a site-specific guideline.

Use of the Weight of Evidence Approach

When conducting ecological risk assessments (ERAs) many pieces of information are often missing, which makes drawing conclusions sometimes difficult. To deal with this, ERA practitioners developed the weight-of-evidence approach (Menzie *et al.* 1996; summarised in Chapman 2001). For many DTA studies the test design and the nature of the organism responses are variable, such that strict imposition of the minimum data requirements used to derive the TVs could result in much valuable site-specific data being discarded. Application of a weight-of-evidence process allows all available site-specific DTA data, alternative laboratory or field endpoints, and different calculation methods to be considered and weighted according to the 11 factors of Menzie *et al.* (1996), which represent how realistic and relevant the data are to the particular site. It is conceivable that this weight-of-evidence approach could be used when considering the other factors discussed in this paper that affect the toxicity of chemicals at specific sites, and it was behind the general approach for PCP and pH effects described earlier.

In the original derivation of the guidelines, it was only possible to consider the data from multiple-species pond and stream experiments for a few chemicals. The available mesocosm data were used to derive additional estimates of trigger values, which were in turn used in a weight of evidence approach to ascertain if the Guideline trigger values were reasonable. If the estimated values and the Guideline TVs were similar, then there is additional confidence that the Guideline trigger value is correct. In contrast, if the values were different it would decrease the confidence in the Guideline trigger value.

Esfenvalerate was the only chemical for which there were sufficient multiple species pond studies that fully satisfied the criteria for accepting mesocosm data (see Warne 2001) to permit the calculation of a trigger value. The pond NOEC values of 0.01 µg/L (4 separate measures), 0.18, 0.2 and 0.25 µg/L were used to derive the Guideline TV of 0.001 µg/L. If the laboratory acute data (obtained in the absence of suspended and natural organic matter) were used, application of a factor to the lowest of these (0.07 µg/L) would have resulted in a trigger value of 0.0007 µg/L, not greatly different from the field-derived figure. This may suggest that suspended and dissolved materials are not significantly ameliorating toxicity of esfenvalerate.

Mesocosm data were available for some other chemicals but could not be used for deriving trigger values, usually because of insufficient replication and/or numbers of treatments, and insufficient breadth of taxa. The NRA (1997) reviewed a number of aquatic microcosm and mesocosm studies for atrazine with different combinations of species and test endpoints. Most studies only reported LOEC values of between 50 and 300 µg/L but seven NOEC values were considered for the derivation of the atrazine trigger value: 3.2, 5, 17.5, 20, 20, 20 and 80 µg/L, although none of these fully satisfied the criteria for accepting mesocosm data. The lowest figure was a stimulation effect and was not

Table 4. NOEC data (µg/L) used to calculate a site-specific endosulfan trigger value.

Species	NOEC (µg/L)	Time (h[4])	GM of NOEC (µg/L)	Water source	Expt type	Ref.
Fish						
Bidyanus bidyanus	0.98[1]	96	0.98	Me	L	a
Cyprinus carpio	0.04[1]	96	0.04	Me	L	a
Macquaria ambigua	0.12[1]	96		Me	L	a
	0.97	12	0.34	N	M, C	b
Melanotaenia duboulayi	0.98[1]	96	0.98	Me	L	a
Nematolosa erebi	0.08[1]	96	0.08	Me	L	a
Insects						
Atalophlebia australis	0.3	72		N	L[3]	c
	0.3	72		N	L[3]	c
	10	12		N	L[3]	b
	8	12		N	L[3]	b
	4.8	12		N	M,C[3]	b
	6.1	12		N	M,C	b
	4.8	12		N	M,P	b
	6	48		N	L[3]	b
	7	48		N	L[3]	b
	6.1	48		N	M,C[3]	b
	6.1	48		N	M,P	b
	4.2	96	3.70	N	L	
Cheumatopsyche sp	0.2	48	0.35	N	L[3]	c
	0.6	48		N	L[3]	c
Jappa kutera	0.6	72		N	L[3]	c
	0.3	96		N	L[3]	d
	2.4	48		N	L[3]	c
	0.15[2]	96		N	L	d
	6.1	12		N	M,C[3]	b
	4.8	12		N	M,P	b
	1.05	48		N	M,C[3]	b
	1.05	48	1.10	N	M,P	b
Hydraena sp	0.01[2]	72	0.01	N	M,P	e
Triplectides sp	0.01[2]	72	0.01	N	M,P	e
Aquatic community structure	1.45	72	1.45	N	M,P	e
Crustaceans						
Paratya australiensis	0.5	96	0.5	N	M,C	b,f

Each row of figures represents a separate experiment. Intermediate times in a single experiment were not included.
a. Sunderam *et al.* (1992); b. Hose (2000); c. Leonard *et al.* (1999); d. Leonard *et al.* (2001); e. Brooks 1998 (insufficient replication of controls, so not included in final calculation); f. results from S. Wilson, *pers. comm.* 2000.
1. NOECs estimated from LC50/5 (adapted from Van de Plassche *et al.* 1993; as used in ANZECC & ARMCANZ 2000); 2. NOECs estimated from LOEC/2 (adapted as for "1"); 3. Specific size classes as separate experiments; 4. 12 hour exposures (Hose 2000) indicated endosulfan exposure followed by a period (usually 96h) of flow-through with clean water.
GM = Geometric mean of above NOEC data from column 1. L = Laboratory experiments using local water and conditions; M = Mesocosm experiment; C = caged animals; P = Population; Me = Mehi River water; N = Namoi River water.

considered. The next lowest NOEC (5 µg/L) was not very different from the 95%TV (13 µg/L) derived from laboratory data. Atrazine may require further examination, particularly in mesocosm experiments. Similarly for chlorpyrifos, up to 27 mesocosm studies have been reported, but only three produced NOEC figures. The lowest mesocosm NOEC (0.06 µg/L for stream macroinvertebrates after pulse exposure) was not markedly higher than the figure from the chronic laboratory data (0.01 µg/L).

RESEARCH NEEDS

The examples given above cover many of the known quantitative relationships between water quality parameters and toxicity of organic or non-metallic inorganic chemicals, and hence water quality guidelines. There is need for more information on such relationships for many priority chemicals. The gaps in data provide a focus for future research, which in time will create a greater level of understanding of the wider issue of bioavailability, especially of organic chemicals.

SUMMARY

When the guidelines for toxicants are applied to specific sites in Australia and New Zealand, it is unlikely that any two sites or situations will be exactly the same. The optional decision scheme described by Chapman (2001) outlines the general approach for applying trigger values to specific sites. However, at present there are few quantitative relationships available between water quality parameters and the toxicity of organic or non-metallic inorganic chemicals. Where general relationships exist, such as for ammonia and PCP at different pH values, the TVs can be adjusted with confidence. The example with PCP suggests that broad scale adjustments of the TV for water quality parameters may be just as appropriate and less time-consuming than adjusting each data point, at least with large and robust data sets. Until further data can be obtained on other chemicals and for other parameters, users are advised to take the most precautionary approach when making any quantitative adjustments for water quality parameters. In the absence of quantitative information, it may be possible to make qualitative estimates of increased or decreased risk at specific sites. The option of direct toxicity assessment (DTA) is always available, and given the ability of DTA to estimate overall biological effects, an appropriate suite of DTA tests may be cost-effective in many cases. Application of a weight-of-evidence approach has considerable merit, especially where different types of site-specific data provide partial information on different aspects of the effects of the chemical at a specific site.

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