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DIRECT TOXICITY ASSESSMENT (DTA) FOR WATER QUALITY GUIDELINES IN AUSTRALIA AND NEW ZEALAND

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ABSTRACT

Direct toxicity assessment (DTA) involves the assessment of the adverse impacts of complex mixtures of compounds, ranging from concentrated industrial effluents to natural waters, on aquatic organisms. The major benefit of DTA is that it can assess the toxicity of waters, in which the number of unidentified components may number thousands, and their behaviour, or interactions cannot be predicted. Thus, DTA enables a greater understanding of potential impacts to aquatic environments, which in turn aids in the development of environmental protection measures.

The Australian and New Zealand Guidelines for Fresh and Marine Water Quality has recently undergone a major revision, particularly in its philosophical approach. The emphasis is on deriving water quality guideline values that more accurately reflect the particular ecosystem being considered. Thus, site-specific issues and characteristics are of major importance. As such, DTA has been recommended as one of the tools available for developing more relevant guideline values, and for establishing whether aquatic ecosystems are being adequately protected.

This paper discusses a number of important aspects of DTA, including its advantages and limitations, its potential applications and the recommended use of DTA in the revised Water Quality Guidelines. The current status of DTA in Australia and New Zealand is briefly reviewed, and three case studies highlighting the benefits of the approach are presented. Guidance and considerations for the development of DTA test methods are detailed in order to provide managers with an increased awareness of the requirements. Finally, recommendations for carrying out DTA for the purposes recommended in the revised Water Quality Guidelines are presented.

Key words: direct toxicity assessment, whole effluent toxicity testing, water quality guidelines, Australia, New Zealand.

INTRODUCTION

Aquatic toxicity tests are used to detect and assess the potential toxicological effects of chemicals on aquatic organisms (Rand 1995; Giesy and Graney 1989), and have been carried out for over fifty years (Parrish 1985). Data from laboratory, single-chemical and single-species toxicity tests have formed the basis for deriving chemical-specific water quality guidelines for Australia and New Zealand for a number of years. While such toxicity tests have dominated ecotoxicological research, more complex methods also exist, such as multi-species and ecosystem level tests. In addition, toxicity tests can be carried out in the natural environment (*in situ*), and can assess the effects of complex mixtures of compounds, such as effluents and leachates. The assessment of the toxicity of complex mixtures is known as Direct Toxicity Assessment (DTA) or Whole Effluent Toxicity (WET) testing. In Australia and New Zealand, DTA can be a useful technique for water managers to consider when dealing with mixtures of compounds in ambient waters, such as industrial effluents, or for the monitoring of natural waters in general. The revised Australian and New Zealand Guidelines for the Protection of Fresh and Marine Water Quality (ANZECC & ARMCANZ 2000) recommends the use of DTA as just one tool for deriving more relevant site-specific guidelines.

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In preparing this paper, ecotoxicologists from Australia and New Zealand were consulted as to existing protocols, and priority issues of DTA that should be addressed. Such issues included the use of DTA methods for ambient water quality monitoring, and the development of site-specific guidelines. Therefore, this paper discusses the advantages and disadvantages of DTA compared to the more common single chemical toxicity test methods, and the situations in which DTA could be carried out. In addition, it provides an overview of the status of DTA in Australia and New Zealand, and examines several case studies in order to highlight the benefits of this approach to both water managers and industry. Finally, it discusses factors that need to be considered for the development of DTA protocols, and provides guidance and recommendations for DTA programs.

SINGLE CHEMICAL TOXICITY TESTING - BENEFITS AND LIMITATIONS

Like most experimental techniques, single chemical toxicity tests have particular benefits and limitations. Of major benefit is the fact that specific information can be obtained on the overall toxicity of a particular chemical. Such information is utilised for the derivation of water quality guidelines for the protection of aquatic ecosystems. Definitive limits can be set and, assuming there is an analytical detection method for the compound, it can be readily monitored in aquatic environments. In addition, the majority of single chemical toxicity tests are carried out in the laboratory, where effects can be studied under controlled conditions with a limited number of variables (Sprague 1990). Assuming such experiments are carried out correctly, there is a large degree of certainty that the observed effects are caused by the chemical alone. Therefore, for the majority of compounds, single chemical toxicity tests are the most appropriate way of determining their toxicity and hence deriving water quality guidelines.

As an organism will rarely be exposed to just one toxicant in the environment (Sprague 1990), single-chemical toxicity testing is not representative of the situation in the natural environment. In most circumstances, a particular chemical will be present in combination with many other chemicals and interactions may occur which may alter their toxicity. Subsequently, mixtures of chemicals can result in either additive toxicity, greater than additive toxicity (also known as synergism), or less than additive toxicity (antagonism) (Rand 1995). Single-chemical toxicity tests do not account for such factors and the extrapolation of the results to environmental impacts carries much uncertainty. While methods exist for predicting the toxicity of mixtures by utilising data from single chemical toxicity tests (Marking 1977; Warne 1998), they obviously require knowledge of the chemical components and their interactions. This is often not the case for complex effluents and waste waters.

While strict control of all variables bar the few of interest is usually considered a benefit in laboratory experiments, it has also been recognised as their major limitation (Rand 1995). Manipulation of environmental factors can be incorporated into a laboratory toxicity test (eg. water hardness for metals), but they cannot simulate all aspects of the natural environment. Other limitations include the use of a constant toxicant concentration (which is often not the case in natural systems), the use of a limited range of standard test organisms, and the need to use optimal culture/living conditions for test organisms (again a potentially uncommon occurrence in the environment). Therefore, it is difficult to be sure that effects observed in such experiments will resemble those in the natural environment.

Various methods have commonly been utilised to address and minimise some of the limitations of single-chemical laboratory toxicity tests. They include: the use of application or safety factors, a practice widely used in the derivation of water quality guidelines world-wide, although questioned more recently (Chapman *et al.* 1998); focussing on data from the most sensitive species tested; and the use of alternative statistical estimates, such as the EC5 as opposed to the EC50. In addition, there has been a growing trend towards making the actual assessment of the effects of aquatic contaminants more realistic, such as the development of more relevant toxicity test protocols, including multi-species and laboratory microcosm tests, outdoor mesocosm tests, and tests for determining the toxicity of complex mixtures, such as effluents, and urban and industrial run-off waters. The following section discusses the concepts behind, and the advances in, the toxicity testing of complex mixtures of compounds.

DIRECT TOXICITY ASSESSMENT

Direct toxicity assessment (DTA) or whole effluent toxicity (WET) testing, as it is termed in the United States, is by no means a new development in the field of ecotoxicology. Hart *et al.* (1945) published a paper emphasizing that the importance of the toxicity of mixtures has long been recognised. The types of mixtures that can be assessed include urban run-off waters, sewage discharges, mining waste waters, agricultural run-off waters containing pesticides and increased nutrient loads from fertilisers, any type of

industrial effluent, or any combination of compounds which occurs in, or is likely to enter the environment, for which the toxicity is unknown. This can also include the assessment of the toxicity of ambient (natural) waters that receive contaminant inputs. Therefore, DTA differs from single chemical toxicity testing in that the combined effects of a number of compounds of unknown identity and concentration are assessed, as opposed to the effects of just one chemical. However, the DTA approach has generally been adapted from conventional toxicity testing approaches, using the same methods, species selection and extrapolation to receiving waters (Mount 1986).

Grothe and Johnson (1996) stated that the primary aim of WET testing (and thus DTA) is to ensure that waste water releases into the aquatic environment do not harm aquatic life. In fact, this can also be broadened to account for (semi) natural changes in water quality, such as eutrophication, hypoxia, salinisation, etc. It aims to do so by measuring the overall discrete toxicity of a mixture of compounds, and is generally not concerned with individual components. As with other methodologies, DTA has its benefits and limitations, and these are discussed below. A more comprehensive review of the topic is provided by de Vlaming and Norberg-King (1999).

Benefits of direct toxicity assessment

DTA has become an important tool for ecotoxicologists, for assessing the toxicity of complex waste waters and receiving, or ambient waters, where the number of components may often number thousands, and are unlikely to be fully identified. The effects of such complex mixtures cannot usually be predicted by determining the toxicity of the individual components, which typically change with time and are often not fully known (Holdway 1992). DTA provides an integrative measure of the aggregate/additive toxicity of chemicals within a mixture (de Vlaming *et al.* 2000), and thus accounts for interactions between compounds. Therefore, it more closely resembles the situation in the natural environment than single-chemical testing.

Other benefits of DTA techniques include: they provide a direct measure of toxicity and bioavailability; they are reliable qualitative predictors of biological community impacts (Waller *et al.* 1996; de Vlaming and Norberg-King 1999; de Vlaming *et al.* 2000); they can provide an early warning capability so that management actions can be implemented to minimise ecosystem impacts (van Dam *et al.* 1998a, de Vlaming *et al.* 2000); and they can be performed relatively quickly and at less cost compared to other biological monitoring procedures (de Vlaming and Norberg-King 1999; de Vlaming *et al.* 2000).

Limitations of direct toxicity assessment

Although considered as being more representative of the natural environment, DTA has also come under criticism. In successfully assessing the toxicity of a mixture as a whole, DTA fails to identify the toxic components of a mixture (Jop *et al.* 1991). While they might be obvious for a simple waste containing only a few well-defined contaminants, for the majority of cases there will be too many chemical components to easily identify those that are toxic. Identification of the toxic component(s) of a waste water is an essential step for industry to address this toxicity problem and to improve treatment technology, and DTA alone cannot provide this. However, it should be recognised that DTA is only one step in an overall assessment of a discharge or water quality in general (Chapman 1995, 2000). Specific methods for identifying the toxic components of effluents (toxicity identification evaluation, TIE) do exist and are discussed briefly, below.

Due to the variable nature of waste waters and effluents and the fact that their compositions are usually unknown, it may be difficult to obtain a representative sample of the mixture (Mount 1986). Therefore, *one-off* testing of a chemical mixture will give little meaningful information if the representativeness of the sample is unknown. Repeated testing or continuous monitoring is desirable, but may not be cost-efficient.

There also exist several technical problems in the use of DTA, which most likely stem from the fact that the field is still in its infancy, even in the United States. While several of these are mentioned below, it is likely that improvements in testing procedures will eventually resolve many of them. In Australia, there is a lack of existing standard protocols for the preparation of effluents for DTA (J Stauber, CSIRO, pers comm), although standard protocols have been developed in North America (Environment Canada 1990a, b; 1992a, b, c, d; US EPA 1993, 1994a, b, 1995a). Aspects of effluent preparation include collection, storage, filtration, dilution, adjustment of physico-chemical parameters, and aging. Aging is particularly important, as it relates to the persistence of chemicals, and hence toxicity at time zero may be very different to that after 48 hours (Mount 1986). In addition, chronic tests may exceed the optimum effluent holding storage time, in which case the experiment is conducted using different samples at different times. While this will possibly increase the environmental realism of the assessment, it will also potentially add to the variation and increase the uncertainty (Pifher and Egan 1989). Filtration is also a vital step, with microorganisms (eg. bacteria) and macroorganisms (eg. predatory copepods) having the potential to interfere with toxicity if effluents are not filtered correctly (Grothe and Johnson 1996). However,

filtration can also significantly reduce the environmental realism of an effluent sample.

The selection of appropriate DTA methods is also a contentious issue. In the United States, standard methods are utilised to determine effluent toxicity, and this has been criticised by industry (Pifher and Egan 1989). There has been a call for environmentally representative testing, however, this also has been subjected to criticism. The relative advantages of standard and site-specific DTA are discussed as a separate point, below.

Standard versus site-specific DTA

Although a discussion on the pros and cons of standard and site-specific toxicity testing applies to all forms of toxicity testing, only DTA is considered here. The basic differences between standard and site-specific toxicity testing lie in the methodologies. As the name implies, standard toxicity tests were developed to standardise the processes used by ecotoxicologists, to enable comparison of the results of experiments conducted on different effluents from similar industries, and to encourage the generation of scientifically sound data. Tests are usually carried out using standard organisms, a standard synthetic water, and standard test conditions (eg. pH, temperature, dissolved oxygen), duration and endpoints. An important criterion in the selection of suitable standard species is sensitivity to a wide range of toxicants; combined with the use of application factors and conservative exposure conditions, this makes standardised tests more likely to be overprotective to the aquatic ecosystem of interest (Chapman 2000; Chapman *et al*. 1998). However, standardised toxicity tests are generally not representative of the local environment, and hence have limited applicability for making conclusions about potential local environmental effects. That is, a significant effect from a test does not necessarily mean there is a problem in the receiving water (Pifher and Egan 1989), while a negative result may not necessarily mean the waste water is not impacting on the receiving ecosystem.

Recently, there has been increasing interest in site-specific test protocols, in which the tests are designed according to the environmental conditions of interest (ie. the environment that an effluent is, or will be entering into). Organisms local to the area are chosen as test species, while the local receiving water (upstream from the effluent source, or from a *clean* reference site) is used as the control and dilution water. In addition, conditions such as test duration and test endpoints can be manipulated to best represent the likely nature of exposure to a particular effluent. As with DTA versus single-chemical testing, site-specific testing is more representative of the environment of interest than standardised testing. However, it is not generally possible to make comparisons with other effluents if, for example, different species are used, and the water chemistry of the receiving waters differs. In addition, utilising upstream water as dilution water may result in the introduction of other variables, such as background toxicity from compounds introduced further upstream (Pifher and Egan 1989). Finally, the use of natural water as control and dilution water increases the complexity of the testing and presents further problems related to background effects, while treating and filtering natural waters may also alter the toxicity of contaminants (Ruffier 1996).

Another approach to site-specific testing is *in situ*, or in-stream testing, where organisms are exposed to the receiving water or waste water in the actual environment (eg. fish kept in cages). Effects are monitored in comparison to organisms kept either upstream of the contaminant source, or in a designated reference or *clean* area. Examples of such methodologies are provided by Humphrey *et al.* (1999) and Maltby *et al.* (2000).

Field validation of laboratory results is a useful approach for ultimately assessing environmental impacts, and also for determining confidence in predicting impacts from laboratory studies. For example, Eagleson *et al.* (1990) documented the results of 43 comparisons between laboratory DTA and in-stream surveys and found that there was 88% agreement between the laboratory and field based methods. In addition, several more recent studies have concluded that DTA (or WET) procedures, if used properly, are reliable qualitative predictors of aquatic population impacts (Waller *et al*. 1996; de Vlaming and Norberg-King 1999). However, other studies have demonstrated poor correlation between laboratory and field effects (Clements and Kiffney 1994; Sarakinos and Rasmussen 1998), although there does not appear to be a unidirectional trend of laboratory bioassays over- or underestimating field effects (Chapman *et al*. 1998). Thus, field validation of laboratory experimentation is an extremely difficult objective to meet, and is rarely possible (Chapman 2000).

Depending on the objective of an investigation, a decision must be made as to which type of DTA method, standard or site-specific, should be adopted. For the purposes of Australian water managers, who generally oversee specific geographical regions and are concerned with local water quality, site-specific DTA is likely to be the most appropriate approach.

Toxicity Identification Evaluation

Toxicity identification and evaluations (TIE) are a set of toxicity assessment procedures developed and modified to identify toxic components of effluents or contaminated natural waters quickly and cheaply (Jop *et al.* 1991; Maltby *et al.* 2000). They involve manipulating and fractionating the effluent or natural water, and subsequently carrying out toxicity tests to separate toxic from non-toxic components (Burkard and Ankley 1989). TIE methodologies have been extensively developed in North America (Jop *et al.* 1991; Norberg-King *et al.* 1991, 1992; Durhan *et al.* 1993; Mount and Norberg-King 1993), and can be undertaken following DTA if necessary. Some TIE methodologies have also been developed in Australia (Manning *et al.* 1993; Pablo *et al.* 1996; Bailey *et al.* 2000a, b). TIE is becoming an increasingly important tool, however, guidance for its use is not within the scope of the revised Guidelines for Fresh and Marine Water Quality. A TIE case study is described below, however, the reader is referred to the above-mentioned papers for detailed information and guidance.

APPLICATIONS OF DTA

Philosophically, it would be ideal if DTA could be carried out on every discrete mixture of chemicals that is known to enter the aquatic environment, however, this is most likely impossible. In addition, state and federal government legislation will also determine the priorities and uses for testing, and whether DTA can actually be utilised as a regulatory, and therefore, enforcement tool. In the United States there has previously been considerable disagreement between government and industry as to whether WET testing, with its associated limitations should be utilised to determine compliance with enforcement requirements (Pifher and Egan 1989; Moore *et al.* 2000a). However, WET testing has now become an important component of many industrial and municipal National Contaminant Discharge Elimination System (NPDES) permits throughout the United States (Grothe *et al.* 1996). It should be noted that DTA has often proved beneficial to industry and it should be seen as a useful tool, not as a hindrance.

A summary of the potential applications of DTA is given in Table 1. Specific industries, or processes, where discrete complex effluents or waste waters are released into aquatic ecosystems should initially be targeted for DTA. These could include waste waters from mining, pulp and paper, sewage treatment and power generation industries, as well as urban run-off waters. If a water quality monitoring program of a receiving water already exists, this should be carried out in conjunction with DTA of the specific discharge, as well as DTA of the receiving water. Due to the large number of chemicals likely to be present in many waste waters, it is possible that some compounds of concern could be missed if only suspected priority contaminants are measured. The use of DTA overcomes this limitation as it integrates the toxicity of all the compounds in a complex mixture. Alternatively, the measurement of priority contaminants may also assist DTA results by identifying the toxic component(s). Toxicity testing of waste waters prior to their release into the aquatic environment aims to prevent contamination of a receiving water with waste water that is toxic to aquatic life, and also to monitor the performance of waste water treatment facilities. In addition, it allows the determination of site-specific waste water dilution and release rates.

It has been recognised that the majority of industries that discharge effluents into receiving waters are relatively small (Mount 1986). It may be that their numbers are such that carrying out DTA on all the effluents would not be feasible, both economically and scientifically, or that the contaminant sources are difficult to define. In such situations, laboratory or *in situ* DTA of the receiving waters would represent the most appropriate option.

In addition, many contaminants enter aquatic waterways over a broad spatial scale, with no particular point source, making assessment of their specific toxicity difficult. Again, laboratory or *in situ* toxicity testing of the receiving waters can be utilised in such situations. Alternatively, experiments can be specifically designed to catch run-off waters for laboratory DTA, or mixtures such as mining leachates can be prepared in the laboratory, following standard methods, for laboratory testing. Essentially, if an area is suspected as being polluted, DTA can either be carried out *in situ*, or in the laboratory using collected water samples, and using either representative clean water from elsewhere (eg. upstream), or synthetic water that is characteristic of the region, as dilution water.

The monitoring of ambient waters in a manner described above could well be the most relevant application of toxicity testing methods to water managers in Australia and New Zealand. This includes the use of DTA for determining whether naturally-elevated background levels of inorganic compounds represent a risk to aquatic life, or whether other site-specific characteristics, such as salinity, pH and dissolved organic carbon ameliorate or increase the toxicity of particular compounds or mixtures of compounds. In addition, assessing the bioavailability and toxicity of one or more chemicals under site-specific conditions (ie. local species, local dilution

Table 1. *Summary of the applications of direct toxicity assessment (DTA). Note the concurrent monitoring of the receiving water utilising water quality guidelines for single chemicals for the majority of applications.*

	Application	Types of DTA and associated monitoring
1.	Major industry discharging waste water into water body (eg. mining, pulp and paper, sewage treatment, power generation).	Laboratory or *in situ* DTA of pre-release waste water. Laboratory or *in situ* DTA of receiving water. Monitoring water quality of receiving water using existing water quality guideline values for single chemicals. Biological monitoring.
2.	Series of known, or unknown minor sources of contaminants entering a water body.	Laboratory or *in situ* DTA of receiving water. Monitoring water quality of receiving water using existing water quality guideline values for single chemicals. Biological monitoring.
3.	Suspected polluted run-off water or leachate entering water body (eg. mining leachates, agricultural run-off waters).	Laboratory or *in situ* DTA of collected run-off water or leachate. Laboratory DTA of laboratory-prepared leachate. Laboratory or *in situ* DTA of receiving water. Monitoring water quality of receiving water using existing water quality guideline values for single chemicals. Biological monitoring.
4.	Background ambient water concentration for a chemical exceeds Water Quality Guideline value.	Laboratory or *in situ* DTA of ambient/receiving water. Monitoring water quality of receiving water using existing water quality guideline values for single chemicals. Biological monitoring.
5.	Future industrial development likely to release waste/run-off water into water body.	Laboratory DTA of pilot plant or simulated (laboratory-prepared) effluent as part of risk assessment. Monitoring water quality of receiving water using existing water quality guideline values for single chemicals pre-development. DTA as per Application 1, once development is complete.
6.	Assessment of the bioavailability or toxicity of a chemical in waters appropriate to a specific site (eg. site specific evaluation of water quality guideline).	Laboratory DTA. *In situ* or mesocosm tests may be appropriate, with controls on test effluent. Biological and chemical monitoring associated with chemical use.

water), to derive a site-specific trigger value may also be a common application of DTA. The use of DTA for such purposes has been recommended for site-specific situations in the revised Guidelines for Fresh and Marine Water Quality (ANZECC & ARMCANZ 2000). The emphasis of the revised Water Quality Guidelines is on developing guideline 'trigger' values for toxicants, and through a hierarchical decision framework, providing the water manager/regulator with a means of modifying the values based on site-specific characteristics. The philosophy and methodology of the guideline 'trigger' value derivation process and the hierarchical decision framework is outlined in Warne *et al.* (this issue). DTA is recommended as a latter step in the decision framework, if the bioavailable concentration of a toxicant still exceeds the modified, site-specific trigger value. However, it is emphasised that DTA can be undertaken at any point during the decision framework (see Chapman (2001) for details). Additionally, DTA of a particular chemical in local waters using laboratory or mesocosm tests may provide useful site-specific information on the guideline value for that chemical, by providing information on toxicity or bioavailability in the local waters.

DTA can also be used for predictive ecological risk assessment, whereby the toxicity of simulated effluents, produced from pilot or benchtop plants, is assessed. Following plant construction, DTA can be employed to monitor the toxicity of the effluent or waste water, and receiving water, as described above.

In summary, DTA should be seen as a useful tool for monitoring the toxicity of complex effluents entering aquatic ecosystems, and where the basic measurement of suspected priority chemicals might be insufficient to monitor and ultimately protect the aquatic environment. In addition, DTA can be used as a regular monitoring tool for pre-release waste waters, as a means of early intervention of waterway contamination. Where contaminant sources are difficult to define, DTA can still be used to assess the toxicity or quality of the natural receiving waters, as had been recommended in the revised Water Quality Guidelines (ANZECC & ARMCANZ 2000). Similarly, DTA can be used to derive site-specific trigger values by assessing the toxicity of one or more chemicals under site-specific conditions. Finally, another major use of DTA could be within a predictive risk assessment framework, as a tool for assessing the effects of simulated effluents from proposed developments. However, in being used as a predictive tool, their primary role is that of screening; together with other appropriate tools, they can form a useful predictive risk assessment approach (Chapman 2000).

CASE STUDIES OF DTA

The following three case studies are presented in order to highlight the uses of DTA and their benefits to both regulatory water managers and industry managers. Hall and Golding (1998) provide a series of specific examples of DTA applications in New Zealand.

Mount Lyell Remediation Research and Demonstration Program (MLRRDP)

Mining and ore processing, over 100 years, at the Mount Lyell mine lease at Queenstown, western Tasmania, resulted in the deposition of more than 100 million cubic metres of tailings, slag and topsoil in the Queen and King Rivers and Macquarie Harbour, causing severe environmental damage (Supervising Scientist 1996). The Mount Lyell Remediation Research and Demonstration Program (MLRRDP) was initiated, and undertaken jointly by the Supervising Scientist and the Tasmanian Department of Environment and Land Management (DELM), in order to determine the environmental impact of metal release from the mining operation, and define a remediation plan (Supervising Scientist 1996).

Part of the program included a study to assess the potential biological impact of elevated levels of copper (Cu) in Macquarie Harbour as a result of mining operations. This was achieved by using toxicity tests to determine Cu concentrations in Macquarie Harbour waters that would not be detrimental to aquatic life (Stauber *et al.* 1996). Bioassays were carried out to assess the toxicity of both ionic Cu (ie. single-chemical toxicity testing), and either filtered or unfiltered Macquarie Harbour water (ie. DTA) on two marine algal species (*Nitzschia closterium* and *Dunaliella tertiolecta)*, an amphipod (*Allorchestes compressa)* and juvenile flounder (*Rhombosolea tapirina*). The effects of salinity on toxicity were also assessed. Tests ranged from a 1-hour enzyme inhibition bioassay for *D. tertiolecta*, to a 27-day growth and survival bioassay for *A. compressa*.

Ionic Cu showed significant effects on juvenile flounder and algal population growth at concentrations as low as 4 and 5 µg/L, respectively (Stauber *et al.* 1996). Total dissolved Cu in collected Macquarie Harbour waters ranged from 10-42 µg/L, with 6-24 µg/L estimated to be potentially bioavailable (Stauber *et al.* 1996). These figures suggested that dissolved Cu concentrations in Macquarie Harbour should be highly toxic to local marine/estuarine organisms. However, DTA of the harbour water revealed that there were no significant effects on algal growth, amphipod and juvenile flounder survival, or osmoregulation and copper accumulation in juvenile flounder, indicating that much of the dissolved Cu was not present in bioavailable forms (Stauber *et al.* 1996). It was suggested that the dissolved Cu was mostly bound to iron, manganese and aluminium oxides/hydroxides, limiting its bioavailability. While some adverse effects of Macquarie Harbour water were observed for *D. tertiolecta, A. compressa and R. tapirina*, they were not major, and occurred at higher Cu concentrations than those at which toxicity of ionic Cu was observed (Stauber *et al.* 1996).

The study estimated the maximum acceptable Cu concentration in Macquarie Harbour waters to be between 10-20 µg/L, requiring a two- to four-fold reduction of dissolved copper from present levels. However, if the toxicity of Macquarie Harbour waters had been predicted only by extrapolating laboratory results of ionic Cu toxicity to measured Cu levels in the Harbour, actual toxicity would have been grossly overestimated. By also testing actual Macquarie Harbour waters, Cu was found to be largely non-toxic, due most likely to its limited bioavailability (Stauber *et al.* 1996).

Toxicity of effluent and effluent components from a newsprint mill

As part of New South Wales Environment Protection Authority (NSW EPA) requirements, the Murray-Darling Freshwater Research Centre (MDFRC) carried out DTA on Australian Newsprint Mills' (ANM) waste water at Albury, NSW. Laboratory testing was carried out on both river water, below ANM's point of discharge, and on treated waste water. In addition to the monitoring carried out by the MDFRC, several other studies have been carried out which are related to the potential environmental impacts of this waste water source on the River Murray. Together, they form a useful case study emphasising the benefits of DTA.

The chelating agent, diethylenetriamine pentaacetic acid (DTPA) is a significant component of the effluent produced by ANM's recycling and de-inking facility, present at concentrations of up to approximately 10 mg/L (Richardson *et al*. 1994). Extensive research conducted on the toxicity of DTPA to the freshwater cladoceran, *Daphnia carinata*, found that reproduction was significantly impaired at concentrations as low as 2 to 5 mg/L DTPA, while in ultra-soft water (<5 mg/L Ca), growth was reduced at 1 mg/L DTPA (van Dam *et al*. 1998b). These effects suggested that levels of DTPA in the waste water were potentially harmful to aquatic organisms.

In 1991, the Key Centre for Applied and Nutritional Toxicology (RMIT, Melbourne, Victoria) used crimson-spotted rainbowfish (*Melanotaenia fluviatilis*) to assess the toxicity of existing newsprint mill effluent, and a simulated de-inking plant effluent, which contained over 110 mg/L DTPA. The study was part of an environmental impact statement on a proposed ANM newsprint de-inking and recycling facility (now in operation). The simulated de-inking plant effluent caused no mortality of larval rainbowfish over 24 h, while 2.9% effluent (highest dilution tested) resulted in no adverse mortality or growth effects over a 14 day exposure period (Holdway 1996). In addition, 2.9% effluent had no effect on the hatchability of rainbowfish eggs (Holdway 1996). The apparent lack of toxicity of the simulated effluent which contained >110 mg/L DTPA was attributed to the selective complexation of DTPA with iron. Supporting this, other studies have demonstrated that the toxicity of DTPA to both *D. carinata* and *M. fluviatilis* is greatly reduced when complexed with iron (van Dam 1997; van Dam *et al*. 1996; 1998b).

As stated above, the MDFRC was contracted by ANM to monitor the toxicity of various stages of the actual waste water as well as the downstream receiving water of the River Murray (MDFRC 1994)[1]. Treated de-inking process effluent exhibited no acute or chronic toxicity to *D.carinata*, while chronic exposures of 100% treated ANM waste water to eastern rainbowfish (*M. duboulayi*) and freshwater crayfish (*Cherax destructor*) also showed no apparent toxicity or bioaccumulation of metals (H.King, MDFRC, pers comm).

Therefore, chronic toxicity of uncomplexed DTPA alone, to *D. carinata* occurred at concentrations regularly present in ANM waste water, however, the same waste water exhibited no, or very little toxicity.

Sydney Water Corporation Hawkesbury-Nepean River STPs Toxicity Assessment Program

A direct toxicity assessment (DTA) and toxicity identification evaluation (TIE) study of effluent from 17 Sewage Treatment Plants (STP) that discharge to the Hawkesbury-Nepean River catchment STPs was undertaken by Sinclair Knight Merz Ecotoxicology Laboratory and EVS Environment Consultants. This toxicity assessment and identification study is one of the largest programs of this type in the world.

Effluent samples were tested using the 48-hour acute and 7-day chronic test with the freshwater cladoceran *Ceriodaphnia dubia* and the 96-hour algal growth test with the unicellular green alga *Selenastrum capricornutum* (Bailey *et al*. 2000a). If toxicity in the effluent was observed, a TIE study was undertaken to identify the cause(s) of the toxicity.

The results of the TIE studies showed that some STPs exhibited acute and chronic toxicity on each occasion. Organophosphorus (OP) pesticides were responsible for toxicity to *C.dubia* in all samples where the identity of the toxicant was confirmed. The pesticides identified as causing toxicity were diazinon and chlorpyrifos, with chlorfenvinphos also being identified as causing toxicity at one STP. Diazinon and chlorpyrifos were identified in STP effluents across the catchment, and were at times, present together at concentrations sufficient to cause toxicity to *C. dubia*. Fewer STP effluents exhibited toxicity to *S. capricornutum*. TIE studies demonstrated that toxicity to *S.capricornutum* was caused either by competition for nutrients by other algal species in some samples, and by unidentified organic compounds which dissipated before the toxicant(s) could be characterised.

In summary, where each STP had approximately 20 chemicals identified as chemicals-of-concern in the initial ecological risk assessment, only diazinon and chlorpyrifos were confirmed as exhibiting toxicity in the effluent. Chlorfenvinphos was not identified as a chemical-of-concern by the risk assessment process.

[1]ANM now re-uses all treated process water for the irrigation of pine plantations, with the River Murray receiving only cooling water (H. King, MDFRC, pers. comm.).

Sydney Water has undertaken a sewer survey study to determine the source, frequency and concentration of pesticides entering the sewer system. These data were used to design a public education program with the aim of reducing pesticide discharges to the sewer system.

GUIDANCE FOR SITE-SPECIFIC DTA IN AUSTRALIA

Direct toxicity assessment is still in its infancy in Australia and New Zealand compared to Europe and the United States. However, several institutions have developed protocols and carried out a significant amount of research utilising DTA for both government and industry. While the United States Environment Protection Agency (US EPA) has developed standard acute and chronic WET testing procedures for over ten freshwater and marine species (US EPA 1993, 1994a, b, 1995a), development of protocols in Australia has generally been on a regional or site-specific basis. This is almost certainly a result of the absence of a formal national approach to DTA development (in contrast to the US), with specific institutions developing protocols to suit particular regions and purposes. In contrast, New Zealand has recently completed the development of standard testing protocols (Hall and Golding 1998). Table 2 provides an overview of the major DTA protocols and programs that have been developed both in Australia and New Zealand, without entering into specific details, or attempting to cover all toxicity testing programs or associated research. The aim of this section is to discuss the major factors that must be considered when developing DTA protocols in Australia.

While the development of protocols based on site-specific conditions, or on an individual case basis, is seen as being advantageous for Australian conditions, it is highly desirable that guidelines are followed for the design of such tests, in order to maintain scientifically sound research standards. An *ad hoc* approach to the development of site-specific DTA protocols would likely result in large variations in the quality of data gathered, and ultimately in a loss of confidence in the use of site-specific approaches. The success of any DTA application is dependent on the ability of the toxicity test methods to deliver robust and relevant data at reasonable cost to both the water manager and the discharger (Environment Agency 1996). While a sufficient number of DTA-specific toxicity bioassays have now been developed in Australia to most likely be relevant to many locations and situations (Table 2), it remains important to know or understand the types of issues that need to be considered when developing site-specific protocols. The following issues are of major importance: test species selection, dilution water selection, nature of the contaminant, test methodology, test/biological endpoints, statistical endpoints, and quality assurance/quality control. Within each of these factors, there are further considerations that must be taken into account, and these are dealt with, below.

Test species selection

When selecting appropriate species for site-specific toxicity testing purposes, several criteria should be considered. Firstly, and ideally, the species should have regional relevance, ie. it should be an important component of the receiving system of interest. However, a species which has economic relevance (eg. fisheries, tourism) may also be a useful test species (Evans *et al.* 1996). Test species should also exhibit relative sensitivity to the contaminant being assessed, although this is often difficult to determine. In addition, identification of sensitive life stages of a species (usually early life stages) is desirable, while successful and efficient laboratory culturing must also be considered when selecting an appropriate test species. The use of wild organisms in toxicity tests is possible, but results may be difficult to interpret, as the previous condition of the organisms will be unknown, and intra-specific variation will most likely be high (US EPA 1993). It is also essential that test organisms represent different trophic levels. The general consensus is that organisms from at least three trophic levels should be tested. For example, a primary producer (eg. aquatic plant or alga), a herbivore (eg. cladoceran), and a vertebrate predator (eg. fish) would represent an adequate range of trophic levels. Evans *et al.* (1996) also considered similarity to Northern Hemisphere species when selecting appropriate north-west Australian species to assess the effects of compounds produced by the oil and gas industry, in order to assist in comparisons to similar overseas programs. In addition, Evans *et al.* (1996) selected test species that could be utilised for both acute and chronic toxicity assessments, further rationalising their program. Obviously, such considerations are specific to the issue being investigated.

Finally, if organisms are chosen which have a wide geographical distribution (eg. southern coast of Australia), the protocols can be sufficiently standardised so as to allow for comparisons between contaminants and/or different laboratories, while still retaining site-specific characteristics (Evans *et al.* 1996). For Australia, it may be beneficial to develop distinct DTA protocols for both temperate and tropical species, to account for major latitudinal differences. This has already been achieved to some extent, with specific DTA programs being established for fresh and marine waters in both

Table 2. Summary of major toxicity bioassays used in Australia and New Zealand for Direct Toxicity Assessment (DTA) purposes.

Test organism	Test duration (acute/chronic)	Test endpoint	Organisation/Institution	References
Marine				
Australia				
Bacterium, *Vibrio fischeri*	15 min (acute)	luminescence	CAAC, NSW EPA	Stauber *et al.* (1994a, b)
	22 h (chronic)	luminescence	NSW EPA	Stauber *et al.* (1994a, b)
	30 min sediment (acute)	luminescence	AWT ES&T	Stauber *et al.* (1994a, b)
Alga (Diatom), *Nitzschia closterium*	72 h (chronic)[1]	cell division rate	CAAC, NSW EPA	Stauber *et al.* (1994a, b);
	96 h (chronic)[1]	cell division rate	CAAC, NSW EPA	Stauber *et al.* (1994a, b)
Green Alga, *Dunaliella tertiolecta*	1 h (acute)	enzyme inhibition (ß-D-galactosidase)	CAAC	Peterson and Stauber (1996)
	72 h (chronic)[1]	cell division rate	CAAC	Stauber *et al.* (1994b)
Alga, *Isochrysis* sp.	72 h (chronic)[2]	cell division rate	CUT, SKM	Evans *et al.* (1996); Tsvetnenko *et al.* (1996)
Brown macroalga, *Hormosira banksii*	2.5 h (sub-chronic)[1,3,7]	fertilisation	NSW EPA, MAFRI	Stauber *et al.* (1994a, b)
Brown macroalga, *Ecklonia radiata*	24 or 48 h (sub-chronic)[4,7]	zoospore germination, germination tube length	AWT ES&T, UniSA	Bidwell *et al.* (1998)
Green macroalga, *Ulva lactuca*	72 h (sub-chronic)[7]	gametophyte development	AWT ES&T, NSW EPA	AWT ES&T (1996a)
Sea urchin, *Heliocidaris tuberculata*	1 h (sub-chronic)[1,7]	fertilisation	AWT ES&T, SKM	AWT ES&T (1996a), Simon and Laginestra (1997)
	80 min. (sub-chronic)[1,7]	fertilisation	NSW EPA	AWT ES&T (1996a)
	72 h (sub-chronic)[7]	embryo development	AWT ES&T, NSW EPA, SKM	AWT ES&T (1996a)
Sea urchin, *Heliocidaris erythrogamma*	6 d (sub-chronic)[5]	settlement	AWT ES&T	King (1999)
Sea urchin, *Centrostephanus rodgersii*	1 h (sub-chronic)[5,7]	fertilisation	AWT ES&T	King (1999)
	68 h (sub-chronic)[5,7]	larval development	AWT ES&T	King (1999)
Gastropod, *Polinices conicus*	24–96 h (acute)	survival, burying behaviour	RMIT	Gulec and Holdway (1996)
Doughboy scallop, *Mimachlamys asperrima*	48 h (sub-chronic)[1,6,7]	larval abnormality	NSW EPA, SKM	Krassoi *et al.* (1996)
Sydney rock oyster, *Saccostrea commercialis*	48 h (sub-chronic)[6,7]	larval abnormality	NSW EPA, SKM	Krassoi (1996)
	44 h (sub-chronic)[5,7]	larval development	AWT ES&T	King (1999)
Mussel, *Mytilus edulis planulatus*	48 h (sub-chronic)[5,7]	larval development	AWT ES&T	King (1999)
Copepod, *Gladioferens imparipes*	96 h (acute)[2]	survival	CUT	Evans *et al.* (1996); Tsvetnenko *et al.* (1996)
Amphipod, *Allorchestes compressa*	96 h (acute)	survival	AWT ES&T, RMIT, SKM	AWT ES&T (1996a); Gulec and Holdway (1996)
	7–28 day (chronic)			
Amphipod, *Hyalella crassicornis*	96 h (acute)[6]	survival	NSW EPA, SKM	Everett (1997)
Amphipod, *Victoriopisa australiensis*	10 d (sub-acute)[6]	survival	NSW EPA, SKM	Everett (1997)
Amphipod, *Corophium* sp.	10 d (acute)[6]	survival	AWT ES&T, NSW EPA, SKM	Hyne and Everett (1998)
	14d (sub-acute)[6]	growth/survival	NSW EPA	Hyne and Everett (1998)
Prawn, *Penaeus monodon*	96 h (acute)[2]	survival	CUT, NSW EPA, SKM	Evans *et al.* (1996); Tsvetnenko *et al.* (1996)

Table 2. Summary of major toxicity bioassays used in Australia and New Zealand for Direct Toxicity Assessment (DTA) purposes. ***(Continued).***

Test organism	Test duration (acute/chronic)	Test endpoint	Organisation/Institution	References
Tasmanian blenny, *Parablennius tasmanianus*	96 h (acute)[1]	Survival	AWT ES&T, Uni Tas	Stauber *et al.* (1994a)
	21 day (chronic)[1,3]	larval development	AWT ES&T, Uni Tas	Stauber *et al.* (1994a)
Sand flathead, *Platycephalus bassensis*	96 h[1]	hepatic EROD induction	RMIT	Brumley *et al.* (1996)
New Zealand				
Alga, *Dunaliella tertiolecta*	72 h (chronic)	growth inhibition	NIWA	Hall and Golding (1998)
Sand dollar, *Fellaster zelandiae*	36 h (sub-chronic)[7]	larval development	NIWA	Hall and Golding (1998)
Sand flounder, *Rhombosolea plebeia*	96 h (acute)	juvenile survival	NIWA	Hall and Golding (1998)
Freshwater				
Australia				
Green alga, *Chlorella protothecoides*	72 h (chronic)[1]	cell division rate	CAAC	Stauber *et al.* (1994b)
Green alga, *Selenastrum capricornutum*	72 h or 96-h (chronic)[1]	cell division rate	AWT ES&T, CAAC, SKM	Stauber *et al.* (1994b); Bailey *et al.* (2000 a)
Green alga, *Chlorella* sp. (two different species)	48 or 72 h (chronic)	cell division rate	CAAC	Franklin *et al.* (1998); Franklin *et al.* (2000)
Green Hydra, *Hydra viridissima*	96-h (acute)	survival	RMIT	Pollino and Holdway (1999); Mitchell and Holdway (2000)
	96 h (chronic)	population growth rate	***eriss***	Markich and Camilleri (1997)
	7 day (chronic)	Population growth rate	RMIT	Pollino and Holdway (1999); Mitchell and Holdway (2000)
Pink Hydra, *Hydra vulgaris*	96 h (acute)	survival	RMIT	Pollino and Holdway (1999)
	7 days (chronic)	population growth rate	RMIT	Pollino and Holdway (1999)
Snail, *Amerianna cumingii*	96 h *in situ* (acute) (acute/chronic)	reproduction, juvenile survival	***eriss***	Humphrey *et al.* (1995)
Marine				
Australia				
Bacterium, *Vibrio fischeri*	15 min (acute)	luminescence	CAAC, NSW EPA	Stauber *et al.* (1994a, b)
	22 h (chronic)	luminescence	NSW EPA	Stauber *et al.* (1994a, b)
	30 min sediment (acute)	luminescence	AWT ES&T	Stauber *et al.* (1994a, b)
Alga (Diatom), *Nitzschia closterium*	72 h (chronic)[1]	cell division rate	CAAC, NSW EPA	Stauber *et al.* (1994a, b);
	96 h (chronic)[1]	cell division rate	CAAC, NSW EPA	Stauber *et al.* (1994a, b)
Green Alga, *Dunaliella tertiolecta*	1 h (acute)	enzyme inhibition (ß-D-galactosidase)	CAAC	Peterson and Stauber (1996)
	72 h (chronic)[1]	cell division rate	CAAC	Stauber *et al.* (1994b)
Alga, *Isochrysis* sp.	72 h (chronic)[2]	cell division rate	CUT, SKM	Evans *et al.* (1996); Tsvetnenko *et al.* (1996)
Brown macroalga, *Hormosira banksii*	2.5 h (sub-chronic)[1,3,7]	fertilisation	NSW EPA, MAFRI	Stauber *et al.* (1994a, b)
Brown macroalga, *Ecklonia radiata*	24 or 48 h (sub-chronic)[4,7]	zoospore germination, germination tube length	AWT ES&T, UniSA	Bidwell *et al.* (1998)
Green macroalga, *Ulva lactuca*	72 h (sub-chronic)[7]	gametophyte development	AWT ES&T, NSW EPA	AWT ES&T (1996a)
Sea urchin, *Heliocidaris tuberculata*	1 h (sub-chronic)[1,7]	fertilisation	AWT ES&T, SKM	AWT ES&T (1996a), Simon and Laginestra (1997)
	80 min. (sub-chronic)[1,7]	fertilisation	NSW EPA	AWT ES&T (1996a)
	72 h (sub-chronic)[7]	embryo development	AWT ES&T, NSW EPA, SKM	AWT ES&T (1996a)
Sea urchin, *Heliocidaris erythrogamma*	6 d (sub-chronic)[5]	settlement	AWT ES&T	King (1999)

Table 2. Summary of major toxicity bioassays used in Australia and New Zealand for Direct Toxicity Assessment (DTA) purposes. **(Continued).**

Test organism	Test duration (acute/chronic)	Test endpoint	Organisation/Institution	References
New Zealand				
Alga, *Selenastrum capricornutum*	72 h (chronic)	cell division rate	NIWA	Hall and Golding (1998)
Cladoceran, *Ceriodaphnia dubia*	48 h (acute), 7 days (chronic)	survival	NIWA	Hall and Golding (1998); Hickey (1989)
Cladoceran, *Daphnia carinata*	24 h (acute)	survival	NIWA	Hickey (1989)
	14 days (chronic)	reproduction		
Amphipod, *Paracalliope fluviatilis*	48 h (acute)	survival	NIWA	Hall and Golding (1998)
Common bully, *Gobiomorphus cotidianus*	96 h (acute)	survival	NIWA	Hall and Golding (1998)

1. Developed as part of the National Pulp Mills Research Program (NPMRP).
2. Developed for the North-West shelf oil and gas industry.
3. Developed in conjunction with CAAC, MAFRI and the Department of Aquaculture, University of Tasmania.
4. Developed in conjunction with UniSA, the Department of Environmental Management, Edith Cowan University, and the Department of Biological Sciences, Victoria University of Technology.
5. Developed in conjunction with the School of Biological Sciences, Sydney University and AWT ES&T, Sydney. Whilst yet to be published externally, the protocols have been fully developed and validated using strict developmental guidelines. They are expected to be published externally in the near future (C. King, University of Sydney, pers. comm.).
6. Developed in conjunction with the Centre for Ecotoxicology, NSW Environment Protection Authority and University of Technology, Sydney.
7. **Such sub-chronic toxicity tests can be used in combination with chronic test data to derive site-specific trigger values.**

Acronyms: AWT ES&T, Australian Water Technologies, Environment, Science and Technology, Sydney, NSW; CAAC, Centre for Advanced Analytical Chemistry, Lucas Heights, NSW; CUT, Curtin University of Technology, Perth, WA; ***eriss***, Environmental Research Institute of the Supervising Scientist, Jabiru, NT; MAFRI, Marine and Fisheries Research Institute, Queenscliff, Vic.; MDFRC, Murray-Darling Freshwater Research Centre, Albury, NSW; NIWA, National Institute for Water and Atmospheric Research, Hamilton, New Zealand; NSW EPA, New South Wales Environment Protection Authority, Gore Hill, NSW; RMIT, Royal Melbourne Institute of Technology, Melbourne, Vic; SKM, Sinclair Knight Merz – Ecotoxicology laboratory, Sydney, NSW; UniSA, University of South Australia, Adelaide, SA.

tropical (Hyne *et al.* 1996;Tsvetnenko *et al.* 1996) and temperate (Stauber *et al.* 1994a; Bailey *et al.* 2000a) zones.

Dilution water selection

The choice of dilution water may have a profound effect on toxicity test results. Dilution water serves two primary functions: i) it is used as control water for the test; and ii) it is combined with the contaminant to provide different contaminant concentrations for testing (Burton *et al.* 1996). Therefore, the dilution water should possess characteristics that closely resemble those of the receiving water so a realistic assessment of toxicity can be obtained. In the case of a river system, dilution water should be collected from upstream of the contaminant source, but still represent the quality of the receiving water with which the contaminant mixes (Burton *et al.* 1996). For other water bodies such as lakes, dilution water could be collected from an undisturbed region, assuming the lake is large, or from another lake, preferably nearby and with similar physico-chemical characteristics. Utilising water from elsewhere as dilution water also applies in the case of a heavily polluted river, where a clean upstream water source is difficult to identify. For marine DTA, dilution water would preferentially be collected from a nearby, but non-impacted area. An advantage of carrying out DTA on simulated effluents from proposed developments, is that the actual receiving water proposed to receive contaminant inputs can be used as dilution water. The only uncertainty in this case is that of temporal variations associated with the receiving waters.

Synthetic water can be used as the diluent instead of natural water, however, again it must be representative of the receiving water (Burton *et al.* 1996). Standard synthetic waters are available which can to a certain extent represent particular natural waters (eg. in hardness, pH, temperature, conductivity, dissolved oxygen, trace metal composition). However, it may be desirable to develop synthetic water that is specifically based upon the characteristics and constituents of the receiving water of interest. Again, however, temporal variations in receiving water characteristics, and therefore water quality, may complicate the development of representative synthetic waters. It should be noted that the development of a receiving water-specific synthetic water would likely be a time consuming process, and possibly only feasible in long, on going programs dealing with a specific water body.

Nature of the contaminant

It is useful to have some prior knowledge about the process which produces the test chemical mixture, the manner in which it is released and its major components. Illustrating this, an effluent produced from a paper mill that used chlorine bleaching contained periodic high spikes of chlorine. Acute toxicity tests using a cladoceran and fish indicated no toxicity, however, in-stream benthic monitoring indicated severe habitat degradation (J Bidwell pers comm). This discrepancy was due to the fact that the sampling design did not account for the periodic spikes of the primary toxicant, chlorine. Increasing the sampling frequency or sampling duration, or coordinating sampling with changes in the process that are known to result in changes to the effluent, are all ways of improving sampling designs.

In considering the nature of a mixture, issues such as transportation and storage methods also need to be considered, as components may degrade or interact with other components over time. US EPA (1993) recommended that no more than 36 h elapse between sample collection and first use in a test, and stipulated that at no time should more than 72 h elapse. Transportation and storage times of mixtures for toxicity assessment should be minimised where possible. Pre-treatment of effluent or natural water prior to a toxicity test is another issue that needs to be considered. As mentioned under the Limitations of DTA, above, filtration, dilution, and adjustment of physico-chemical parameters may potentially alter the toxicity of an effluent/natural water. Ideally, the effects of such treatments on effluents or natural waters needs assessing, however, each mixture would need to be considered on its own merits. An established DTA program for pre-release waste water from Ranger uranium mine in the Northern Territory recommends filtration of the water through a 10 µm filter to remove any large particles and wild zooplankton (Hyne *et al.* 1996). In the absence of more information on the procedures for and effects of such treatments, any water preparation methods should be kept as consistent as possible and all steps clearly described.

Test methodology

The test method will vary depending on the objective of the test. Initially, decisions are required as to whether toxicity of a pre-release mixture or a receiving water is to be assessed, whether acute and/or chronic toxicity is to be assessed, and whether laboratory and/or *in situ* toxicity testing is to be carried out. Often, results of a mixture's toxicity are required rapidly, and this may also influence the type of methods used. Acute toxicity tests are generally shorter, but regularly used endpoints such as lethality tend to be less sensitive than chronic, sub-lethal endpoints. Chronic, or at the very least sub-chronic toxicity tests are now carried out on certain organisms in relatively short time periods, and therefore may be more appropriate. For example, algal bioassays can generally assess contaminant effects on chronic parameters such as population growth over approximately 72 h (3 days; Stauber *et al.* 1994b), while similar parameters can be assessed using *Hydra*, over 96 h (4 days; Hyne *et al.* 1996). Similarly, sub-chronic assessment of the effects of contaminants on reproduction in particular species of cladocerans takes only 6 days. US EPA conducts 7-day toxicity tests using fish to estimate chronic toxicity (US EPA 1994a, b, 1995a).

Laboratory toxicity test systems can be either of a static, static-renewal, or flow-through design. Without entering into details, the selection of the test design will depend upon the objective of the test, available resources, test organism requirements and characteristics of the contaminant (US EPA 1995b). In static tests, organisms are exposed to the same mixture for the duration of the test. In static-renewal tests, test solutions are replaced at defined time intervals, usually every 24 or 48 h. Flow-through test designs can be divided into two major types; i) the mixture is pumped directly from the source, through a dilutor system and to the test chambers; or, ii) grab or composite samples are taken from the source, placed in a holding tank and pumped continuously through a dilutor system to the test chambers. While being more representative of the situation in the receiving environment, flow-through systems are costly and difficult, especially at off-site locations (US EPA 1995b). However, where on-site facilities exist, flow-through systems utilising continuous sampling are useful, and allow *in situ* testing. Another method of *in situ* testing involves the placement of caged organisms, usually fish, in the receiving water, downstream or at increasing distance from the contaminant source. This has been achieved with considerable success in Europe, using freshwater mussels to measure long-term water quality of heavily polluted rivers (Kramer *et al.* 1989). In general, static-renewal toxicity test systems are an acceptable compromise to flow-through conditions, while being considered superior to static systems, except where continuous exposure is not appropriate to the problem being studied.

Test / biological endpoints

It is essential when selecting an appropriate test species that an appropriate biological endpoint can be measured. The choice of endpoint will often determine the test duration (Burton *et al.* 1996). The majority of acute toxicity tests use lethality as the test endpoint, and generally run from 2 to 4 days. In the case of small invertebrates, such as cladocerans, lethality is often

replaced by immobility as the test endpoint, as death is difficult to distinguish. Such endpoints, although generally less sensitive than most sub-lethal endpoints, clearly indicate an adverse effect at the individual level, and most likely represent an effect at the population level, which is ultimately the extrapolation being drawn from such studies. Identification of more sensitive, sub-lethal effects that can also predict, with confidence, effects at the population level provide a more comprehensive and realistic assessment of impacts on aquatic life in receiving waters. Growth and reproduction are the two most common types of sub-lethal endpoints assessed, with the latter often being a more reliable indicator of adverse effects in the environment (OECD 1992). However, in some test species, such as cladocerans, reproduction is dependent upon adequate growth, therefore making growth a suitable endpoint for predicting adverse effects. Reproduction can be expressed in various forms, depending on the type of test being conducted, and the test species. For algal toxicity tests, reproduction is generally expressed as the population growth rate (Stauber *et al*. 1994b). For invertebrates such as cladocerans, it can be expressed as the total number of offspring per adult (Hyne *et al*. 1996; van Dam *et al*. 1996) or the intrinsic rate of population increase (r; van Leeuwen *et al*. 1985). For fish it can be expressed as the number of eggs produced per female, the numbers of fertilised eggs produced, or even egg hatchability (van Dam *et al*. 1999). Survival can also be a useful indicator of chronic toxicity, if the test duration is extended, however, this is often impractical and costly. The US EPA use short term toxicity tests (4 to 7 days) to estimate the chronic toxicity of effluents and receiving waters, which include growth, reproduction and survival as test endpoints (US EPA 1994a,b, 1995a).

Recently, more subtle endpoints have been investigated for potential use in DTA. These include the use of biomarkers such as the mixed function oxidases (MFOs) and immunotoxicological endpoints. MFOs have the disadvantage that while they may be suitable indicators of contaminant exposure, they are difficult to relate to adverse effects, or toxicity. Aquatic immunotoxicology is a new and relatively poorly understood discipline, and is yet to be considered a suitable endpoint for DTA. Another parameter that may deserve attention is that of feeding inhibition. Allen *et al*. (1995) and more recently, Orchard *et al*. (2002) have demonstrated that short-term tests assessing feeding inhibition in cladocerans, can be extrapolated to chronic reproductive effects. Similarly, short term toxicity tests on adult cladocerans (1 reproductive instar or ~ 24(48 h) may indicate reproductive effects that can also be extrapolated to population level impacts (Baird *et al*. 1991). Such rapid tests may serve as useful screening bioassays for waste waters and natural waters.

Statistical endpoints

There are two major approaches to statistics for DTA; i) hypothesis testing, and ii) point estimation, and there is currently considerable debate over which is more appropriate. This issue extends beyond DTA, and into most other areas of ecotoxicology and ecological risk assessment. Chapman *et al*. (1996a) and Denton and Norberg-King (1996) discuss the relative pros and cons of both types of approaches in relation to toxicity testing. Only a brief overview is presented here.

Hypothesis testing is primarily concerned with comparing a series of two or more concentrations, typically serial dilutions, to control conditions (ie. absence of the contaminant). Generally, such tests identify the highest concentration of a dilution series that does not differ significantly from the control condition, known as the *no-observed-effect concentration* (NOEC) (Chapman *et al*. 1996a). It should be noted that hypothesis testing need not be restricted to the estimation of the NOEC alone, but it is generally the most common statistical estimate (Chapman *et al*. 1996a). Point estimation calculates the concentration associated with a specified level, or percentage of change (p) from that observed under control conditions, generally known as the effective concentration (ECp) (Chapman *et al*. 1996a). It allows the estimation of concentrations that would cause different magnitudes of responses, such as a 50% reduction in growth (EC50), or a 10% reduction in reproduction (EC10). The effective concentration can also be referred to as the lethal concentration (LC) when lethality is the endpoint.

DTA statistics have relied almost exclusively on hypothesis testing to date, although acute toxicity experiments generally utilise point estimation for the generation of EC50s or LC50s. The major advantages and disadvantages of both techniques are outlined below, however, for a more detailed review, refer to Chapman *et al*. (1996a). The major advantages of hypothesis testing for DTA are that it is a well suited technique for comparing a control treatment with a particular concentration of contaminant, the statistical computations involved are well known and generally straight-forward, and it is easier to directly compare present studies with previous research that has relied on hypothesis testing. The major disadvantage of hypothesis testing is that the calculation of the major statistical estimates, the NOEC and LOEC (lowest-observed-effect concentration), can only be concentrations used in the experiment. As experiments

are often conducted using serial dilutions (eg. 0.1, 1, 10 and 100% contaminant), there are significant concentration gaps for which the effects are unknown, although they will generally not be greater than an order of magnitude in size. Chapman *et al.* (1996b) provide a useful warning against the use of NOECs for regulatory use.

In deriving water quality guidelines for single chemicals, the traditional approach has been to apply a safety factor to the NOEC for the most sensitive species tested, to account for any uncertainties, including the possibility of more sensitive species existing in the environment. A modification, or elaboration of hypothesis testing techniques and the use of safety factors, is that of statistical extrapolation. This approach is recommended for the derivation of water quality guidelines in Australia and New Zealand, and is discussed in detail by Warne (1998; 2001).

Briefly, the approach, modified from Aldenberg and Slob (1993), involves fitting the most appropriate distribution from the Burr Type III family of distributions to all available NOEC data from different species for a compound, to derive an estimated concentration that should protect at least x% of the species in the environment (Fox 1999; Shao 2000). The percentage, x, can vary according to the level of protection afforded to the aquatic ecosystem of interest, with the current water quality guidelines usually recommending a 95% or 99% level of protection (ANZECC & ARMCANZ 2000). It is argued that by utilising all the toxicity data, a more confident estimate of *safe* concentrations is obtained.

The major advantage of point estimation for interpreting DTA data stems from the above-mentioned disadvantage of hypothesis testing. Point estimation considers the response of organisms at every concentration by determining a concentration-response relationship and estimating where effects of a particular magnitude will occur. As a result, ECps are not restricted to being one of the test concentrations, as they are estimated from the concentration-response curve that is fitted to the data (Chapman *et al.* 1996a). Different levels of effect can be estimated (eg. EC5, or EC50) depending on the objective of the study, or what is considered biologically or ecologically significant. However, care must be taken to test concentrations that accurately cover the range of organism response; too large spacing of concentrations in the regions where the effect is occurring will introduce bias into the estimation of ECps (Chapman *et al.* 1996a). There are many models that can be used to generate concentration-response relationships, however care must also be taken to fit appropriate models. To emphasise this, Moore and Caux (1997) used several regression-based models to evaluate 198 toxicity data sets, and found that greater than 80% of the data sets did not produce a single adequate model fit. However, by careful experimental design, this problem can be somewhat alleviated (Moore and Caux 1997). It has been found that depending on the type of data set, estimates of effects below the 10% level can often be model dependent and have large confidence intervals associated with them (Moore and Caux 1997). Noppert *et al.* (1994) recommended that if an ECp value was chosen to replace the NOEC, either a 5% or 10% level should be chosen, although never a level below 5%.

Chapman *et al.* (1996a) suggested that hypothesis testing should be continued to be utilised for DTA until alternative techniques are shown conclusively to be superior statistical tools for estimating safe levels of mixtures in the aquatic environment. However, it was also suggested that the NOEC gradually be replaced by an ECp estimation, and that both values always be reported during the transition period (Denton and Norberg-King 1996). This approach appears to be increasingly adopted.

Quality assurance / quality control

Adequate quality assurance/quality control (QA/QC) measures are required for DTA in order to minimise inter- and potentially more importantly, intra-laboratory and intra-test variability in the protocols (Ruffier 1996). Of major concern, is variability caused by i) analyst experience and ii) test organism health/condition (Burton *et al.* 1996). The former affects proper implementation of test procedures and interpretation of the generated data (Burton *et al.* 1996), while the latter concern will affect test variability through variable organism responses. Some of the major factors affecting test variability and potential solutions to overcome them have been the subject of many papers (eg. DeGraeve *et al.* 1991, 1992; Burton *et al.* 1996; Warren-Hicks *et al.* 2000; Moore *et al.* 2000a, b; Markle *et al.* 2000).

An appropriate QA program will incorporate QC parameters such as performance standards for test validity, reference toxicant records, adequate training documentation, dilution water quality/chemistry monitoring, proper equipment maintenance, proper record-keeping and attention to test organism health (Burton *et al.* 1996; Chapman *et al.* 1996a). Implementation of such measures will help control variability and maintain and/or improve overall results.

Routine reference toxicity testing is one of the best ways to evaluate QA/QC. Laboratories should monitor the calculated endpoints as well as the control treatment mean response for survival, growth and reproduction (Burton *et al.* 1996). While the use of nominal concentrations of the reference toxicant is generally adequate, intermittent analysis of toxicant concentrations is recommended (Burton *et al.* 1996).

As analyst experience, or inexperience, is such a large contributor of variability, it is highly recommended that all DTAs are carried out by specifically trained and equipped personnel or organisations. In addition, it is equally important that there is as little as possible deviation from the established methods without adequate reason, and without being fully documented and justified. A final means of ensuring quality is the implementation of a regular and independent audit component to the QA/QC program.

RECOMMENDATIONS FOR CONDUCTING DTA IN AUSTRALIA

Recommended protocols

A number of DTA-specific test protocols have been developed in Australia as part of major programs undertaken solely for the purpose of the development of such tests. As a result, the methods have been subjected to extremely rigorous quality assurance, while Standard Operating Procedure (SOP) manuals exist for all of them. Therefore, it is recommended that test protocols developed under these programs are used for DTA purposes where possible, assuming they are relevant to the situation and location. The three major programs were; i) the National Pulp Mills Research Program (NPMRP); ii) The Curtin University ecotoxicology program, and; iii) the ***eriss*** ecotoxicology program. The recommended test protocols are as follows, and are also outlined in Table 2:

National Pulp Mills Research Program

- Marine diatom (*Nitzschia closterium*) 72 h population growth test (chronic)
- Marine alga (*Dunaliella tertiolecta*) 72 h population growth test (chronic)
- Brown macroalga (*Hormosira banksii*) 2.5 h fertilisation test (acute)
- Freshwater alga (*Selenastrum capricornutum*) 72 h population growth test (chronic)
- Freshwater alga (*Chlorella protothecoides*) 72 h population growth test (chronic)
- Sea urchin (*Heliocidaris tuberculata*) 1(2 h fertilisation test (acute)
- Doughboy scallop (*Chlamys asperrima*) 48 h larval abnormality test (acute)
- Tasmanian blenny (*Parablennius tasmanianus*) 96 h larval survival test (acute)
- Tasmanian blenny (*P. tasmanianus*) 21 day larval development test (chronic)

Curtin University Ecotoxicology Program

- Marine alga (*Isochrysis* sp.) 72 h population growth test (chronic)
- Marine copepod (*Gladioferens imparipes*) 96 h survival test (acute)
- Marine prawn (*Penaeus monodon*) 96 h survival test (acute)

***eriss* Ecotoxicology Program**

- Green hydra (*Hydra viridissima*) 96 h population growth test (chronic)
- Freshwater cladoceran (*Moinodaphnia macleayi*) 3 brood/6 day reproduction test (chronic)
- Purple-spotted gudgeon (*Mogurnda mogurnda)* 96 h larval survival test (acute)

Many of the above-recommended tests will only be relevant in particular locations, while others will have broader relevance. Decisions on which test protocols to use should be based on the geographical location of the waterbody or discharge to be tested, and therefore the relevance of the test species. The tests developed by ***eriss*** are specific for tropical freshwater environments in northern Australia. Similarly, the Curtin ecotoxicology program developed tests specific for western and north-western Australian sub-tropical and tropical marine waters (ie. local species), and thus they are recommended for use in this region. The test protocols developed by the NPMRP have a somewhat broader applicability and the majority of them can probably be used throughout eastern and south-eastern Australia. With the exception of two freshwater algal toxicity tests, the NPMRP test protocols are for marine environments. Therefore, where test protocols for freshwater environments are required, and those developed by ***eriss*** are not applicable (ie. not in the tropics), the following additional protocols are also recommended (see Table 2):

- Freshwater cladoceran (*Ceriodaphnia dubia or Daphnia carinata*) 48 h survival test (acute)
- Freshwater cladoceran (*C. dubia*) 3 brood/7-10 days survival and reproduction test (chronic)
- Eastern rainbowfish (*Melanotaenia duboulayi*) 96 h imbalance test (acute)
- Crimson-spotted rainbowfish (*M. fluviatilis*) 96 h survival test (acute)

In some situations it may be determined that none of the above test species are relevant to the situation/ location. In such an event, other currently existing test protocols may be considered (see table 2), but their

reliability, validity and sensitivity should be thoroughly evaluated prior to their use. If the development of a new test using a new species is required, all the factors discussed in the preceding section should be taken into account. In such a case, it would most likely be appropriate to adapt a currently existing protocol to the new test species. Again, only personnel experienced in the procedures of toxicity test development should carry out such a process. It is important to note that much preliminary work is required before a new test can be used for toxicity assessment purposes.

Minimum requirements for DTA in Water Quality Guidelines

Number of species to be tested

To derive a *High Reliability* trigger value for a single toxicant, chronic toxicity data (ie. NOEC values) must be available from at least five different species representing at least four different taxonomic groups (ANZECC & ARMCANZ 2000; and see Warne 2001). As toxicity testing of single chemicals for the derivation of default water quality guidelines is more or less a once-off process, this is a relatively feasible minimum requirement. However, the toxicity testing of effluents and/or ambient waters is often an on-going process due to their changing characteristics over time. Therefore, DTA of an effluent/ambient water requiring data from 5 species representing 4 taxonomic groups may not be achievable. Nevertheless, the highly regarded *battery*, or *suite* of toxicity tests should still be utilised. The use of five species is still recommended where ever possible, particularly for deriving a site-specific trigger value for a single toxicant (see Warne 2001). Otherwise, for all of the above, it is recommended that a minimum of three species, from three different taxonomic groups and trophic levels be assessed. The frequency of assessment becomes a site-specific issue, and is discussed further, below.

Test design: Number of treatments, replicates, organisms

Each of the above-recommended protocols includes guidance on the appropriate number of test treatments, or concentrations/dilutions, replicates, and animals per replicate. In general, the minimum requirement for the number of test treatments is five plus a control. This is also the number recommended by the US EPA (1993) for WET testing purposes, while in the UK, the Environment Agency (1996) recommend six concentrations plus control. However, in keeping with the recommendations for toxicants, sufficient concentrations should be tested to enable the derivation of a concentration-response relationship. In some cases this may require more than the minimum five treatments plus control.

Replication varies from two to twenty depending on the test protocol, while the number of test organisms per replicate also varies greatly between protocols, but is clearly defined in each case. As the protocols have generally been designed for the toxicity assessment of discharges (ie. effluents/waste waters), there is little advice on minimum requirements for the number of test treatments for ambient water toxicity testing. The US EPA (1993) state that receiving (ambient) water toxicity tests commonly employ two treatments; a control/reference and the undiluted receiving water, but may also consist of a series of receiving water dilutions, diluted with control/reference water. The choice of the above two options for ambient water toxicity testing may depend on the objective of the testing. There should be a minimum of three replicates per treatment in either of the above ambient water testing situations.

Test design: Type of test water delivery/ replacement system

Again, the recommended protocols clearly state whether the tests employ static, static-renewal or flow-through designs. None of the recommended protocols employ *in situ* methods, and it may be that such methods are desired for particular monitoring situations. If such tests are to be performed, the existing recommended protocols could most likely be used with some modifications. In keeping with standard ecotoxicological procedures, flow rates through test chambers must be sufficient to allow 99% molecular turnover every 24 h (Sprague 1973). Alternatively, the *in situ* toxicity testing protocols developed and documented by ***eriss*** can most likely be adapted for specific conditions (Table 2; Boyden *et al.* 1995; Humphrey *et al.* 1995).

Test/biological endpoints

The biological endpoints for assessment are clearly specified in each of the recommended protocols. Importantly, they all represent what can be considered ecologically relevant endpoints, such as population growth rate, reproduction, development, growth, and survival. If new tests are to be developed, such functional endpoints should be chosen, in accordance with the criteria for data acceptability for deriving guideline values for toxicants (Warne 2001).

Statistical endpoints

Statistical endpoints may vary depending on whether an effluent/discharge, or an ambient/ receiving water is being assessed. Again, the recommended protocols generally require calculation of either an ECp value and/ or a LOEC and NOEC value. As stated earlier, in order to confidently estimate an ECp value, a concentration-response relationship must be evident, which requires

an adequate number and range of concentrations to be tested. The ECp values are usually calculated using Probit analysis, Logit analysis, Spearman-Karber analysis, or other appropriate statistical software, with the level of 'p' being determined by the operator or water manager, according to what is considered an acceptable level of effect. As mentioned above, the calculation of an EC50, a commonly estimated parameter, requires the application of a safety factor to determine a *safe* level (usually x0.01; Warne *et al.* this issue), while the calculation of an EC5 or EC10 would require a much lower safety factor, or none at all. For current purposes, it is recommended that the EC50 value be reported. To calculate the LOEC (lowest-observed effect concentration) analysis of variance (or an appropriate non-parametric test if required) and an appropriate *post hoc* analysis (eg. Dunnett's test) is usually used to determine the lowest concentration at which a statistically significant response is observed when compared with the control (ie. the LOEC). The NOEC is represented by the next lowest concentration, being the highest concentration at which no statistically significant response was observed when compared with the control. A statistical alpha ($\propto$) level of 0.05 is usually used for such analyses.

Therefore, in recognition of both the historical dominance of the use of hypothesis testing and the current trend towards the use of point estimation, it is recommended that for all experiments, both an ECp value (most probably the EC50, including its corresponding 95% confidence limits) and a LOEC and NOEC value be reported. An EC5 or EC10 value (with 95% confidence limits) can also be reported if desired, however, it should be recognised that such low effects estimates are yet to become universally accepted statistical endpoints for water quality purposes. In addition, it should be understood that to obtain accurate EC5 and EC10 values requires different concentrations and concentration ranges from EC50s (Chapman *et al.* 1996a).

If five NOEC values can be determined for five different species from at least four taxonomic groups, the statistical extrapolation method can be applied to derive a site-specific trigger value (see Warne *et al.* this issue). If only the minimum of three NOEC values for three species from three taxonomic groups are determined, the assessment factor process is adopted, whereby a safety factor (usually (0.1) is applied to the NOEC of the most sensitive species tested (ie. the lowest NOEC) (ANZECC & ARMCANZ 2000).

Frequency of assessment

The frequency of testing a discharge, or ambient water, would depend on the objective of the testing, and on the nature of the discharge or receiving water. If the discharge is known to be of constant composition, and the receiving water characteristics are well documented and understood, one-off testing may be appropriate. Alternatively, if the discharge composition varies considerably and unpredictably, testing will be required on a more frequent basis (eg. monthly). If a discharge varies according to the process being undertaken, but is constant within that process, or if the receiving water varies seasonally, but is relatively constant within seasons, testing can be carried out whenever such a change is known to occur.

CONCLUSION

Direct toxicity assessment (DTA) of effluents and natural waters has gained increasing acceptance over recent years and has been incorporated into regulation and regulatory agency procedures in a number of countries. Methods and protocols using appropriate regional species have been developed in Australia and New Zealand, with assessment programs utilising a battery of tests including algae, invertebrates and fish.

DTA provides a direct biological measure of the likely effect of an effluent or contaminated waterway on organisms in the ecosystem and is complementary to both chemical-specific guidelines and biological assessments. The option of *in situ* testing, either in on-site laboratories or with caged organisms, can provide a valuable tool in real-time assessments of effluents and natural waters. However, the limitations of DTA also need to be understood and these have been discussed both in the present overview, and elsewhere (Grothe *et al.* 1996, Chapman 2000). Nevertheless, Grothe *et al.* (1996) and Chapman (2000) were supportive of the technical validity and effectiveness of direct toxicity assessment, particularly in effluent-dominated situations.

If used in conjunction with chemical measures and biological assessments, DTA will provide a useful tool in maintaining high water quality in Australia and New Zealand. The above guidelines and recommendations for the use of DTA in Australia (and New Zealand; see Hall and Golding 1998) have been proposed in order to increase the ability of water quality management agencies to assess site-specific water quality, and to promote the further development of consistent and scientifically sound methodologies.

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REFERENCES

Aldenberg T and Slob W. 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. *Ecotoxicol. Environ. Saf.* **25**, 48-63.

Allen Y, Calow P and Baird DJ. 1995. A mechanistic model of contaminant-induced feeding inhibition in *Daphnia magna*. *Environ. Toxicol. Chem.* **14**, 1625-1630.

ANZECC & ARMCANZ (Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand) **2000**. *National water quality management strategy, Australian and New Zealand Guidelines for fresh and marine water quality*. ANZECC & ARMCANZ, Canberra, Australia, July 2000.

AWT ES&T (Australian Water Technologies – Environment, Science and Technology Pty Ltd). 1996a. Toxicity testing of sewage effluent from coastal STPs in the Sydney and Illawarra regions. Report No. 0000461. Report for Sydney Water, NSW.

AWT ES&T (Australian Water Technologies – Environment, Science and Technology Pty Ltd). 1996b. Initial toxicity tests for the Hawkesbury-Nepean sewage treatment plants. Report No. 828R1.A. Report for Sydney Water, NSW.

AWT ES&T (Australian Water Technologies – Environment, Science and Technology Pty Ltd). 1997. Characterisation and validation of risk estimates using aquatic toxicity testing for Sydney-Illawarra region. Report No. 97/85. Report for Sydney Water, NSW.

Bailey HC, Krassoi R, Elphick JR, Mulhall AM, Hunt P, Tedmanson L and Lovell A. 2000a. Whole effluent toxicity of sewage treatment plants in the Hawkesbury-Nepean watershed, New South Wales, Australia, to *Ceriodaphnia dubia* and *Selenastrum capricornutum*. *Environ. Toxicol. Chem.* **19**(1), 72-81.

Bailey HC, Krassoi R, Elphick JR, Mulhall AM, Hunt P, Tedmanson L and Lovell A. 2000b. Application of *Ceriodaphnia* cf. *dubia* for whole effluent toxicity tests in the Hawkesbury-Nepean Watershed, New South Wales, Australia. *Environ. Toxicol. Chem.* **19**(1), 88-93.

Baird DJ, Barber I, Soares AMVM and Calow P. 1991. An early life-stage test with *Daphnia magna* Straus; an alternative to the 21-day chronic test? *Ecotoxicol. Environ. Saf.* **22**(1), 1-7.

Bidwell JR. Wheeler KD and Burridge TR. 1998. Toxicant effects on the zoospore stage of the marine macroalga, *Ecklonia radiata* (C.Ag.) J.Ag. (Phaeophyta: Laminariales). *Mar. Ecol. Progr. Ser.* **163**, 259-265.

Boyden J, Pidgeon R and Humphrey CL. 1995. ERISS protocol for the creekside monitoring of Magela Creek waters. II. Larval black-striped rainbow fish, *Melanotaenia nigrans*, survival test. IR 181, Supervising Scientist for the Alligator Rivers Region, Canberra, ACT, Australia.

Brumley CM, Haritos VS, Ahokas JT and Holdway DA. 1996. Evaluation of biomarkers of exposure to 2-chlorosyringaldehyde and simulated ECF bleached eucalypt pulp effluent. In: *Fate and Effects of Pulp Mill Effluents*, Servos MR, Munkittrick KR, Carey JH and Van Der Kraak GJ (Eds). St Lucie Press, Boca Raton, FL, USA. pp 369-378.

Burkhard LP and Ankley JL. 1989. Identifying toxicants: NETAC's toxicity-based approach. *Environ. Sci. Technol.* **23**, 1438-1443.

Burton GA, Arnold WR, Ausley LW, Black JA, DeGraeve GM, Fulk FA, Heltshe JF, Peltier WH, Pletl JJ and Rodgers JH. 1996. Effluent toxicity test variability: Discussion synopsis. In: *Whole Effluent Toxicity Testing: An evaluation of methods and prediction of receiving system impacts*, Grothe DR, Dickson KL and Reed-Judkins DK. (Eds). SETAC Pellston Workshop on Whole Effluent Toxicity; 1995 Sept 16-25; Pellston, MI Pensacola FL: SETAC Press, pp 131-156.

Chapman GA, Anderson BS, Bailer AJ, Baird RB, Berger R, Burton DT, Denton DL, Goodfellow WL, Heber MA, McDonald LL, Norberg-King TJ and Ruffier PJ. 1996a. Methods and appropriate endpoints: Discussion synopsis. In: *Whole Effluent Toxicity Testing: An evaluation of methods and prediction of receiving system impacts*, Grothe DR, Dickson KL and Reed-Judkins DK. (Eds) SETAC Pellston Workshop on Whole Effluent Toxicity; 1995 Sept 16-25; Pellston, MI Pensacola FL. USA. SETAC Press. pp. 51-82.

Chapman J 2001. The revised Australian and New Zealand water quality guidelines for toxicants: Approach to their derivation and application. *Australas. J. Ecotox*. **7**, 95-108.

Chapman PM. 1995. Bioassay testing for Australia as part of water quality assessment programmes. *Aust. J. Ecol.* **20**, 7-19.

Chapman PM. 2000. Whole effluent toxicity (WET) testing – usefulness, level of protection and risk assessment. *Environ. Toxicol. Chem*. **19**, 3-13.

Chapman PM, Caldwell RS and Chapman PF. 1996b. A warning: NOECs are inappropriate for regulatory use. *Environ. Toxicol. Chem*. **15**, 77-79.

Chapman PM, Fairbrother A and Brown D. 1998. A critical evaluation of safety (uncertainty) factors for ecological risk assessment. *Environ. Toxicol. Chem*. **17**(1), 99-108.

Clements WH and Kiffney PM. 1994. Integrated laboratory and field approach for assessing impacts of heavy metals at the Arkansas River, Colorado. *Environ. Toxicol. Chem*. **13**, 397-404.

de Vlaming V, Connor V, DiGiorgio C, Bailey HC, Deanovic LA and Hinton DE. 2000. Application of whole effluent toxicity test procedures to ambient water quality assessment. *Environ. Toxicol. Chem*. **19**, 42-62.

de Vlaming V and Norberg-King TJ. 1999. A review of single species toxicity tests: Are the tests reliable predictors of aquatic ecosystem community responses? EPA 600/R-97/11. Technical Report. U.S. Environmental Protection Agency, Duluth, MN, USA.

DeGraeve GM, Cooney JD, McIntyre DO, Pollock DL, Reichenbach NG, Dean JG and Marcus MD. 1991. Variability in the performance of the seven-day fathead minnow (*Pimephales promelas*) larval survival and growth test: An intra- and interlaboratory study. *Environ. Toxicol. Chem*. **10**, 1189-1203.

DeGraeve GM, Cooney JD, Marsh BH, Pollock TL and Reichenbach NG. 1992. Variability in the performance of the 7-d *Ceriodaphnia dubia* survival and reproduction test: An intra- and interlaboratory study. *Environ. Toxicol. Chem*. **11**, 851-866.

Denton DL and Norberg-King TJ. 1996. Whole effluent toxicity statistics: A regulatory perspective. Discussion-Initiation Paper 4.1. In: *Whole Effluent Toxicity Testing: An evaluation of methods and prediction of receiving system impacts*, Grothe DR, Dickson KL and Reed-Judkins DK. (Eds). SETAC Pellston Workshop on Whole Effluent Toxicity; 1995 Sept 16-25; Pellston, MI Pensacola FL: SETAC Press. pp 83-102.

Durhan EJ, Norberg-King TJ and Burkhard LP (editors). 1993. Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. Duluth MN: US EPA Office of Research and Development. EPA/600/R-92/080.

Eagleson, KW, Lenat DL, Ausley LW and Winborne FB. 1990. Comparison of measured instream biological responses with response predicted using the *Ceriodaphnia dubia* chronic toxicity test. *Environ. Toxicol. Chem*. **9**, 1019-1028.

Environment Agency. 1996. The application of toxicity-based criteria for the regulatory control of wastewater discharges. Direct Toxicity Assessment National Centre, Environment Agency, West Sussex, UK, 50 pp.

Environment Canada. 1990a. *Biological test method: Reference method for determining acute lethality of effluents to rainbow trout.* Environmental Protection Series, Report EPS 1/RM/13, July 1990. Environment Canada, Conservation and Protection, Ottawa, ON. Amended May 1996.

Environment Canada. 1990b. *Biological test method: Reference method for determining acute lethality of effluents to Daphnia magna*. Environmental Protection Series, Report EPS 1/RM/14, July 1990. Environment Canada, Conservation and Protection, Ottawa, ON. Amended May 1996.

Environment Canada. 1992a. *Biological test method: test of larval growth and survival using fathead minnows.* Environmental Protection Series, Report EPS 1/RM/22, February 1992. Environment Canada, Conservation and Protection, Ottawa, ON. Amended November 1997 and March 1998.

Environment Canada. 1992b. *Biological test method: test of reproduction and survival using the cladoceran Ceriodaphnia dubia*. Environmental Protection Series, Report EPS 1/RM/21, February 1992. Environment Canada, Conservation and Protection, Ottawa, ON. Amended November 1997.

Environment Canada. 1992c. *Biological test method: growth inhibition test using the freshwater alga Selenastrum capricornutum*. Environmental Protection Series, Report EPS 1/RM/25, November 1992. Environment Canada, Conservation and Protection, Ottawa, ON. Amended November 1997 and March 1998.

Environment Canada. 1992d. *Biological test method: fertilization assay using echinoids (sea urchins and sand dollars).* Environmental Protection Series, Report EPS 1/RM/27, December 1992. Environment Canada, Conservation and Protection, Ottawa, ON. Amended November 1997.

Evans LH, Bidwell JR, Spickett J, Rippingale RJ, Tsvetnenko Y and Tsvetnenko E. 1996. *Ecotoxicological studies in North West Australian marine organisms: Executive summary.* Curtin University of Technology, unpublished report, 16 pp.

Everett DA. 1997. Development of amphipod sediment toxicity tests. MSc Thesis, University of Technology Sydney, Sydney, Australia, 113p.

Fox DR. 1999. Setting water quality guidelines – A statistician's perspective. *SETAC News* **19**(3), 17-18.

Franklin N, Stauber J, Markich S and Lim R. 1998. A new tropical algal test to assess the toxicity of metals in freshwaters. Supervising Scientist Report 133, Supervising Scientist, Canberra, Australia, 83 pp.

Franklin NM, Stauber JL, Markich SJ and Lim RP. 2000. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (*Chlorella* sp). *Aquat. Toxicol.* 48, 275-289.

Giesy JP and Graney RL. 1989. Recent developments in and intercomparisons of acute and chronic bioassays and bioindicators. *Hydrobiologia* **188/189**, 21-60.

Grothe DR and Johnson DE. 1996. Bacterial interference in whole-effluent toxicity tests. *Environ. Toxicol. Chem.* **15**(5), 761-764.

Grothe DR, Dickson KL and Reed-Judkins DK (editors). 1996. Whole Effluent Toxicity Testing: An evaluation of methods and prediction of receiving system impacts. SETAC Pellston Workshop on Whole Effluent Toxicity; 1995 Sept 16-25; Pellston, MI Pensacola FL: SETAC Press. 340 pp.

Gulec I and Holdway DA. 1996. Toxicity of oil dispersant, crude oil and dispersed crude oil to a marine amphipod and gastropod. *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Sydney, NSW, O176.

Hall JA and Golding L. 1998. Standard methods for whole effluent toxicity testing: development and application. NIWA Client Report: November 1998, National Institute of Water and Atmospheric Research Ltd, Hamilton, NZ.

Hart WB, Doudoroff P and Greenbank J. 1945. The evaluation of the toxicity of industrial wastes, chemicals and other substances to fresh-water fishes. Atlantic Refining Co. Philadelphia, PA.

Hickey CW. 1989. Sensitivity of four New Zealand cladoceran species and *Daphnia magna* to aquatic toxicants. *New Zeal. J. Mar. Fresh. Res.* **23**(1), 131-137.

Holdway DA. 1992. Control of metal pollution in tropical rivers in Australia. In *Pollution in Tropical Aquatic Systems*, eds DW Connell and DW Hawker, CRC Press, Boca Raton, FL, pp 231-246.

Holdway DA. 1996. Acute and sub-chronic toxicity of a pulp and paper mill effluent and a simulated deinking mill effluent (containing DTPA) to embryo and larval rainbowfish (*Melanotaenia fluviatilis*). *Australas. J. Ecotox.* **2**(1), 17-26.

Humphrey CL, Lewis BF, Brown I and Suggit JL. 1995. ERISS protocol for the creekside monitoring of Magela Creek waters. I. Freshwater snail, *Amerianna cumingii*, reproduction and survival test. IR 180, Supervising Scientist for the Alligator Rivers Region, Canberra, ACT, Australia.

Humphrey CL, Thurtell L, Pidgeon RWJ, van Dam RA and Finlayson CM. 1999. A model for assessing the health of Kakadu's streams. *Australian Biologist* **12**(1) 33-42.

Hyne RV and Everett DA. 1998. Application of a benthic euryhaline amphipod *Corophium* sp. as a sediment toxicity testing organism for both freshwater and estuarine systems. *Arch. Environ. Contam. Toxicol.* **34**, 26-33.

Hyne RV and Wilson SP. 1997. Toxicity of acid-sulphate soil leachate and aluminium to the embryos and larvae of Australian Bass (*Macquaria novemaculeata*) in estuarine water. *Environ. Pollut.* **97**, 221-227.

Hyne RV, Rippon GD, Hunt SM and Brown GH. 1996. *Procedures for the biological toxicity testing of mine waste waters using freshwater organisms.* SSR 110, Supervising Scientist for the Alligator Rivers Region, Canberra, ACT, Australia, 67 pp.

Jop KM, Kendall TZ, Askew AM and Foster RB. 1991. Use of fractionation procedures and extensive chemical analysis for toxicity identification of a chemical plant effluent. *Environ. Toxicol. Chem.* **10**, 981-990.

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

King CK. 1999. The impact of metals and organic pollutants on the development of marine invertebrates from the Cl. Echinoidea and Cl. Bivalvia. PhD Thesis, School of Biological Sciences, University of Sydney, Sydney, Australia.

King H and Baldwin D. 1996. Appendix 1: 1996 Annual report of chemical and biological monitoring for Australian Newsprint Mills Ltd Albury. In *Annual Report of compliance with EPA licence requirements and development consent conditions*. Technical Services Division, Australian Newsprint Mills Ltd Albury Mill, File No. l55-0012 (6), RSC\EPA95RPT, 35 pp.

Korth W, Thomas M, Foster S, McCorkelle G and Bowmer KH. 1995. Toxicity of rice and maize pesticides to *Ceriodaphnia* sp; implications for management of irrigation drainage water in Australia. *Australas. J. Ecotox.* **1**, 55-62.

Kramer, KJ, Jenner HA and de Zwaart D. 1989. The valve movement response of mussels: a tool in biological monitoring. *Hydrobiologia* **188/189**, 433-443.

Krassoi R. 1996. Adaptation of the 48 h larval abnormality test using the doughboy scallop and Sydney rock oyster to the assessment of sediments. *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Royal Australian Chemical Institute, 14-18th July, Sydney, NSW, Oral presentation No. 108.

Krassoi R, Everett D and Anderson I. 1996. *Protocol for using Doughboy scallop Chlamys asperrima (Mollusca: Pectinidae) L. to test the sublethal toxicity of single compounds and effluents.* National Pulp Mills Research Program Technical Report No. 17. CSIRO, Canberra, Australia, 56 pp.

Kumar A and Chapman JC. 1997. A toxicity dataset for developing water quality guidelines for profenofos, an organophosphorus pesticide. *Proceedings of the Australasian Society for Ecotoxicology, 4th Annual Conference*, Brisbane, 17-19th July 1997. Oral presentation No. 62.

Maltby L, Clayton SA, Yu H, McLoughlin, Wood RM and Yin D. 2000. Using single-species toxicity tests, community-level responses, and toxicity identification evaluations to investigate effluent impacts. *Environ. Toxicol. Chem.* **19**(1), 151-157.

Manning TM, Evans JL and Chapman JC. 1993. The development of toxicity identification and evaluation procedures in Australia. *Chemistry in Australia*, August 1993, 398-400.

Markich SJ and Camilleri C. 1997. Investigation of metal toxicity to tropical biota: Recommendations for revision of the Australian water quality guidelines. Supervising Scientist Report 127, Supervising Scientist, Canberra, Australia, 87 pp.

Marking LL. 1977. Method for assessing additive toxicity of chemical mixtures. In *Aquatic Toxicology and Hazard Evaluation, ASTM STP 634*, eds FL Mayer and JL Hamelink. American Society for Testing and Materials, Philadelphia, Pa., pp. 99-108.

Markle PJ, Gully JR, Baird RB, Nakada KM and Bottomley JP. 2000. Effects of several variables on whole effluent toxicity test performance and interpretation. *Environ. Toxicol. Chem.* **19**, 123-132.

Mitchell FM and Holdway DA. 2000. The acute and chronic toxicity of the dispersants Corexit 9527 and 9500, water accommodated fraction (WAF) of crude oil, and dispersant enhanced WAF (DEWAF) to *Hydra viridissima* (green hydra). *Water Research* **34**, 343-348.

Moore DRJ and Caux P-Y. 1997. Estimating low toxic effects. *Environ. Toxicol. Chem.* **16**, 794-801.

Moore DRJ, Warren-Hicks W, Parkhurst BR, Teed RS, Baird RB, Berger R, Denton DL and Pletl JJ. 2000a. Intra- and intertreatment variability in reference toxicant tests: implications for whole effluent toxicity testing programs. *Environ. Toxicol. Chem.* **19**, 105-112.

Moore TF, Canton SP and Grimes M. 2000b. Investigating the incidence of Type I errors for chronic whole effluent toxicity testing using *Ceriodaphnia dubia*. *Environ. Toxicol. Chem.* **19**, 118-122.

Mount DI. 1986. Principles and concepts of effluent testing. In *Environmental Hazard Assessment of Effluents*, eds L Bergman, RA Kimerle and AW Maki. Pergamon Press, Oxford, UK, pp. 61-65.

Mount DI and Norberg-King TJ (editors). 1993. Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. Duluth MN: US EPA Office of Research and Development. EPA/600/R-92/081.

MDFRC (Murray-Darling Freshwater Research Centre). 1994. *Annual report of chemical and biological monitoring for Australian Newsprint Mills Ltd Albury*. MDFRC, Albury, NSW, Australia, 26 pp.

Noppert F, van der Hoeven N and Leopold A. 1994. How to measure no effect - towards a new measure of chronic toxicity in ecotoxicology. Workshop Report,

The Hague, The Netherlands: Netherlands Working Group on Statistics and Ecotoxicology. 44 pp. Copies from BKH Consulting Engineers, PO Box 5094, 2600GB, Delft, The Netherlands.

Norberg-King TJ, Mount DI, Durhan EJ, Ankely GT and Burkhard LP (editors). 1991. Methods for aquatic toxicity identifications: Phase I toxicity characterisation procedures, 2nd edition. Duluth MN: US EPA Office of Research and Development. EPA/600/6-91/003.

Norberg-King TJ, Mount DI, Amato JR, Jensen DA and Thompson JA (editors). 1992. Toxicity identification evaluation: characterisation of chronically toxic effluents, Phase I. Duluth MN: US EPA Office of Research and Development. EPA/600/6-91/005F.

OECD (Organisation for Economic Co-operation and Development). 1992. Report of the OECD workshop on extrapolation of laboratory aquatic toxicity data to the real environment. OECD *Environ. Monographs* No 59, OECD, Paris, France.

Orchard SJ, Holdway DA, Barata C and van Dam RA.2002. A rapid response toxicity test based on the feeding rate of the tropical cladoceran *Moinodophnia macleayi*. *Ecotoxicol. Environ. Saf.* B 52, in press.

Pablo F, Chapman JC and Patra R. 1996. Development of toxicity characterisation (TIE) procedures using Australian *Ceriodaphnia dubia*. *Proceedings of the International Symposium on Environmental Chemistry and Toxicology (InterSECT)*, Sydney, NSW, Oral presentation No. 112 (electronic version only).

Parrish PR. 1985. Acute toxicity tests. In *Fundamentals of Aquatic Toxicology: Methods and Applications*, eds GM Rand and SR Petrocelli. Hemisphere Publishing Corporation, Washington, USA, pp 31-57.

Peterson SM and Stauber JL. 1996. New algal enzyme bioassay for the rapid assessment of aquatic toxicity. *Bull. Environ. Contam. Toxicol.* **56**, 750-757.

Pifher MT and Egan JT. 1989. Biomonitoring and toxics control: The POTW perspective. *Nat. Resour. Environ.* **4**(1), pp 13-15 and 48-49.

Pollino CA and Holdway DA. 1999. The potential of hydra species as standard toxicity test animals. *Ecotoxicol. Environ. Saf.* **43**, 309-316.

Rand GM. 1995. *Fundamentals of Aquatic Toxicology*. 2nd Edition. Taylor and Francis, Bristol, PA.

Richardson DE, Ash GH, Harden PE. 1994. Determination of diethylenetriaminepentaacetic acid in pulp mill effluent by ion-interaction reversed-phase liquid chromatography. *J. Chromatog. A* **688**, 47-53.

Ruffier PJ. 1996. Whole effluent toxicity testing: The municipal perspective. In: *Whole Effluent Toxicity Testing: An evaluation of methods and prediction of receiving system impacts*, eds DR Grothe, KL Dickson and DK Reed-Judkins, SETAC Pellston Workshop on Whole Effluent Toxicity; 1995 Sept 16-25; Pellston, MI Pensacola FL: SETAC Press, pp 38-49.

Sarakinos HC and Rasmussen JB. 1998. Use of bioassay-based whole effluent toxicity (WET) tests to predict benthic community responses to a complex industrial effluent. *J. Aquat. Ecosyst. Stress Recov.* **6**, 141-157.

Shao Q. 2000. Estimation for hazardous concentrations based on NOEC toxicity data: an alternative approach. *Environmetrics* **11**, 583-595.

Simon J and Laginestra E. 1997. Bioassays for testing sublethal toxicity in effluents, using gametes of the sea urchin *Heliocidaris tuberculata*. Technical Report No. 20, National Pulp Mill Research Program, 36 pp.

Sprague JB. 1973. The ABC's of Contaminant Bioassay using Fish. In *Biological Methods for the Assessment of water Quality, ASTM STP 528*, American Society for Testing and Materials, Philadelphia, Pa., pp 6-30.

Sprague JB. 1990. Aquatic toxicology. In: *Methods for Fish Biology*, Schreck CB and Moyle PB. (Eds). American Fisheries Society, Maryland, USA, pp. 491-527.

Stauber JL, Gunthorpe L, Deavin J, Munday B and Ahsanullah M. 1994a. *Validation of new marine bioassays using synthetic treated and untreated bleached eucalypt kraft mill effluents.* National Pulp Mills Research Program Technical Report No. 8. CSIRO, Canberra, Australia, 58 pp.

Stauber JL, Tsai J, Vaughan GT, Peterson SM and Brockbank CI. 1994b. *Algae as indicators of toxicity of the effluent from bleached eucalypt kraft pulp mills.* National Pulp Mills Research Program Technical Report No. 3. CSIRO, Canberra, Australia, 146 pp.

Stauber J, Adam M and Low N. 1996. Toxicity of pesticides to the freshwater alga *Selenastrum capricornutum*. CSIRO Investigation Report for Centre of Ecotoxicology, CSIRO Menai, Australia, 17pp.

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

Supervising Scientist. 1996. Annual Report 1995-96. Australian Government Publishing Service, Canberra, ACT, pp. 78-79.

Tsvetnenko YB, Evans LH and Gorrie J. 1996. *Toxicity of the produced formation water to three marine species.* Curtin University of Technology, Final Report prepared for Ampolex Limited, 18 pp.

US EPA. 1993. *Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms.* Office of Research and Development, U.S. Environmental Protection Agency, EPA/600/4-90/027F.

US EPA. 1994a. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms.* 3rd edition, U.S. Environmental Protection Agency, Cincinnati, OH. EPA-600-4-91-002. July 1994.

US EPA. 1994b. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms.* 2nd edition. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, OH. EPA-600-4-01-003. July 1994.

US EPA. 1995a. *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms.* U.S. Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH. EPA/600/R-95-136. August 1995.

US EPA. 1995b. *National policy regarding whole effluent toxicity enforcement.* Washington DC: US EPA Offices of Regulatory Enforcement and Wastewater Management.

van Dam RA. 1997. Toxicity of the chelating agent, diethylenetriamine pentaacetic acid (DTPA) and its ferric-complex, Fe(III)-DTPA, to two Australian freshwater organisms. PhD Thesis, Royal Melbourne Institute of Technology, Melbourne, Australia, 274 pp.

van Dam RA, Barry MJ, Ahokas JT and Holdway DA. 1996. Comparative acute and chronic toxicity of diethylentriamine pentaacetic acid (DTPA) and ferric-complexed DTPA to *Daphnia carinata*. *Arch.Environ. Contam.Toxicol.* 31, 433-443.

van Dam RA, Camilleri C and Finlayson CM. 1998a. The potential of rapid assessment techniques as early warning indicators of wetland degradation: A review. *Environ.Toxicol.Water Qual.* **13**, 297-312.

van Dam RA, Barry MJ, Ahokas JT and Holdway DA. 1998b. Effects of water-borne iron and calcium on the toxicity of diethylenetriamine pentaacetic acid (DTPA) to *Daphnia carinata*. *Aquat.Toxicol.* **42**, 49-66.

van Dam RA, Ahokas JT and Holdway DA. 1999. Chronic toxicity of diethylenetriamine pentaacetic acid to crimson-spotted rainbowfish (*Melanotaenia fluviatilis*): Effects on reproduction, condition and ethoxyresorufin *O*-deethylase activity. *Ecotoxicol. Environ.Saf.* **43**, 74-82.

Van Leeuwen CJ, Luttmer WJ and Griffioen PS. 1985. The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*: a cadmium example. *Ecotoxicol.Environ.Saf.* **9**, 26-39.

Waller WT, Ammann LP, Birge WJ, Dickson KL, Dorn PB, LeBlanc NE, Mount DI, Parkhurst BR, Preston HR, Schimmel SC, Spacie A and Thursby GB. 1996. Predicting instream effects from WET tests. In: *Whole Effluent Toxicity Testing: An evaluation of methods and prediction of receiving system impacts*, eds DR Grothe, KL Dickson and DK Reed-Judkins, SETAC Pellston Workshop on Whole Effluent Toxicity; 1995 Sept 16-25; Pellston, MI Pensacola FL: SETAC Press. pp. 271-286.

Warne M StJ. (1998). Critical review of methods to derive water quality guidelines for toxicants and a proposal for a new framework. Supervising Scientist Report 135, Supervising Scientist, Canberra, 82 pp.

Warne M StJ and Julli M. in press. NSW EPA protocol for acute and chronic toxicity tests using cladocerans: acute immobilisation test and chronic reproduction impairment test using *Ceriodaphnia dubia*. Environment Protection Authority of NSW.

Warne M St J. 2001. Derivation of the ANZECC and ARMCANZ water quality guidelines for toxicants. *Australas.J.Ecotox.* **7**, 123-136.

Warren-Hicks WJ, Parkhurst BR, Moore DRJ, Teed RS, Baird RB, Berger R, Denton DL and Pletl JJ. 2000. Assessment of whole effluent toxicity test variability: Partitioning sources of variability. *Environ. Toxicol.Chem.* **19**, 94-104.